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Physiological and Biochemical Aspects of Ozone Toxicity to Rainbow Trout (*Salmo gairdneri*)

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WEDEMEYER, G. A., N. C. NELSON, AND W. T. YASUTAKE. 1979. Physiological and biochemical aspects of ozone toxicity to rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 36: 605–614.

An acute toxicity curve for dissolved ozone (O_3) in soft water at 10°C, using 10–13-cm rainbow trout (*Salmo gairdneri*) as the test species was calculated. The 96-h LC50 (95% confidence interval) was 9.3 (8.1–10.6) μg/L. The lethal threshold level was about 8 μg/L mandating that a conservative margin of safety be used if ozone is employed as a fish disease control agent. Death apparently results from massive destruction of the gill lamellar epithelium together with a severe hydromineral imbalance. In partial chronic (3-mo) testing, 2 μg/L caused no significant biological damage while 5 μg/L caused some gill pathological changes and reduced feeding behavior. Accordingly, 2 μg/L is suggested as a provisional maximum safe exposure level, pending completion of life cycle studies. Thus, if ozone-treated water is discharged into the environment, dissolved O_3 should be reduced to at least the 2 μg/L level to minimize adverse impacts on salmonids in receiving waters.

Key words: ozone, fish toxicity, acute ozone toxicity, chronic ozone toxicity, *Salmo gairdneri*

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Nous avons établi une courbe de toxicité aiguë de l'ozone (O_3) dissous dans de l'eau douce à 10°C, utilisant, comme espèce expérimentale, des truites arc-en-ciel (*Salmo gairdneri*) de 10 à 13 cm. La LC50 après 96 h (intervalle de confiance à 95%) est de 9,3 (8,1–10,6) μg/L. Le seuil létal est d'environ 8 μg/L. C'est donc dire qu'il faut une marge de sûreté conservatrice si l'on utilise l'ozone comme agent de contrôle des maladies des poissons. Il semble que la mortalité soit le résultat d'une destruction massive de l'épithélium lamellaire des branchies, couplée à un déséquilibre hydrominéral prononcé. Dans des essais partiellement chroniques (3 mois), 2 μg/L ne causent pas de dommage biologique important, alors que 5 μg/L provoquent certains changements pathologiques dans les branchies et diminuent le comportement alimentaire.

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C'est pourquoi nous suggérons provisoirement une concentration de 2 μg/L comme niveau d'exposition sûr maximal, en attendant que soient terminées des études de cycles biologiques. Si de l'eau traitée à l'ozone est introduite dans l'environnement, on devrait réduire la concentration de O_3 dissous au moins au niveau de 2 μg/L afin de minimiser les effets nuisibles sur les salmonidés habitant ces eaux.

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OZONATION for water treatment has recently been attracting substantial commercial and scientific interest (Blogoslawski and Stewart 1977). It is being used successfully to disinfect potable water (Harris 1974), for sewage effluent treatment (Nebel et al. 1973), and as a replacement biocide for chlorine in power plant cooling systems (Siegrist et al. 1975; Blogoslawski and Stewart 1977; Carlson and Caple 1977). In general, ozone is nonpersistent and its considerable oxidative capacity results in an overall water quality improvement in a wide variety of industrial effluents. However, persistent oxidative by-products have been reported in ozonated seawater (Blogoslawski et al. 1976).

Ozone is also being evaluated for disease control in both freshwater and marine fish cultural systems and as a replacement for malachite green in preventing losses due to *Saprolegnia* during egg incubation (Benoit and Matlin 1966; Conrad et al. 1975; Blogoslawski and Stewart 1977; Wedemeyer and Nelson 1977; Wedemeyer et al. 1978). Ozone disinfection of surface water supplies would allow intensive fish culture under the reduced pathogen loads characteristic of the more desirable spring and well sources. Similarly, ozone treatment of hatchery effluents could improve water quality and help prevent the transmission of exotic pathogens to resident fish populations in the receiving waters. In addition, ozone has considerable potential as an oxidative supplement to biological nitrification in reuse systems (Honn and Chavin 1976).

However, before ozone can be routinely used either for fish disease control or as a replacement for chlorine in water treatment systems, its potential for an adverse environmental impact in terms of acute and chronic toxicity to fishes must be assessed. Unfortunately, little information presently exists. In an early report, Hubbs (1930) suggested that levels as low as 0.030 mg/L were "lethal" to minnows (*Cyprinidae*). However, Arthur et al. (1975) reported toxic levels ~10 times higher to fathead minnows (*Pimephales promelas*) exposed to ozonated sewage treatment effluent. In a preliminary study, fingerling rainbow trout (*Salmo gairdneri*) exposed to 20–40 μg/L O_3 all died within $3\frac{1}{2}$ h (Hugh Sparrow, Dep. Parks Conserv., B.C., personal communication). Rosenlund (1975) reported gill epithelial damage and death of rainbow trout exposed to an estimated 10–60 μg/L O_3. Little else is known.

In the present study, the acute toxicity curve and the 96-h LC50 for rainbow trout were calculated. Extensive physiological tests were performed to help elucidate the cause of death due to ozone exposure. Guidelines for the maximum safe exposure level were then determined on the basis of the amount of biological damage occurring during partial chronic (3-mo) tests.

Materials and Methods

Both the acute and partial chronic (3-mo) toxicity tests were done in a flow-through system. The equipment shown schematically in Fig. 1 was evolved to minimize the well-known difficulties of achieving stable dissolved ozone levels. A Pollution Control Industries model C2P-3C ozone generator fed with −40°C dew point air was worked into a sparger-fed, countercurrent, 2-m water tower as the contact chamber. Air flow and pressure through the ozonater

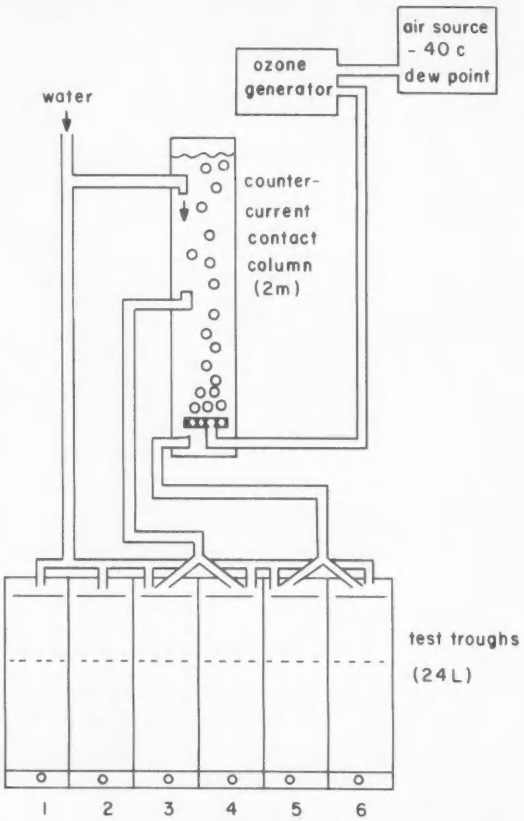


FIG. 1. Schematic diagram of acute and chronic toxicity testing equipment required to control ozone instability problems and consequent fluctuations in exposure levels.

were held constant at 60 mL/min and 1.4 kg/cm², respectively, and the generator voltage adjusted to obtain the desired dissolved ozone level.

The test water characteristics were total hardness, 20 mg/L (as $CaCO_3$); temperature, 10°C; dissolved oxygen, 11 mg/L; pH, 6.8; Cl^-, 2.6 mg/L; Fe^{3+}, 0.2 mg/L; NO_3-N, 0.5 mg/L; turbidity, 3.1 JTU.

Juvenile rainbow trout (10–13 cm) were acclimated to the laboratory water supply for at least 30 d before each test. Standard bioassay procedures were followed (EPA 1975). The Oregon Moist Pellet diet (OMP) was fed daily, but withheld for 24 h before moving fish to the 24-L test troughs. Water flows for each trough were set at 800 mL/min, resulting in a 95% replacement time of 0.5 h and density and flow indices of 1.2 and 2.0, respectively, approximately the same as in the holding tanks. This was done to minimize variability caused by the fact that the fish could not be acclimated to the test troughs before beginning the 96-h LC50 determinations. Unfortunately, ~24 h was required for the ozone residual to come to equilibrium in the system after the ozone generator was turned on.

For the acute toxicity tests, initial 10 fish blood and gill tissue samples were obtained and groups of 60 fish then randomly distributed to duplicate test troughs. Additional 10 fish samples were taken at 4, 24, 48, 72, and 96 h from one of the replicates during the exposure period. Moribund fish were sampled whenever possible. Death was defined as cessation of opercular movement. The troughs were cleaned by siphon daily and the fish were not fed in an effort to minimize fluctuations in ozone demand and to help stabilize exposure levels.

For the partial chronic exposure tests, 35 fish were randomly distributed to duplicate troughs and allowed to adapt for about a week. Initial blood and tissue samples were obtained from 10 fish randomly removed from the six troughs before turning on the ozone generator. Additional five fish blood and tissue samples were taken at 2, 4, 6, 8, 10, and 12 wk. As in the acute exposures, to prevent wide fluctuations in ozone demand, and thus in exposure level, it was necessary to clean the troughs daily and remove mortalities as they occurred. Only a maintenance ration could be fed.

Sampling procedures and analytical methods for both the acute and chronic tests were those outlined in Wedemeyer and Yasutake (1977). McLeay's method (1975) was used for differential blood cell counts except that thrombocytes were included in the total 1000 cells counted per blood smear. Residual ozone was measured spectrophometrically using the *N,N*-diethyl-*p*-phenylenediamine (DPD) procedure modified for 5-cm light path cuvettes. Chlorine standards were used for calibration with the factor 0.69 to convert to ozone (APHA et al. 1975). As with the other ozone assays in common use, this procedure has at best ±15% precision and is not specific for ozone but gives total oxidants present. Water samples were analyzed twice daily during the acute tests and three times a week during the chronic exposures.

Median lethal ozone concentrations were calculated and the resulting toxicity curve determined by the Fisheries Bioassay Laboratory, Montana State University, using a trimmed Spearman–Karber computer program (Hamilton et al. 1971).

Results and Discussion

Acute Toxicity

When tested under controlled conditions, ozone proved to be somewhat more toxic than predicted by the early work of Hubbs (1930) or indicated by the semiquantitative estimates of 10–60 μg/L available from the more recent studies of Rosenlund (1975) and 20–40 μg/L by Sparrow (personal communication). An ozone toxicity curve (Fig. 2) yielded a 96-h LC50 and 95% confidence interval estimate of 9.3 (8.1–10.6) μg/L O_3 with an acute lethal threshold of about 8 μg/L. This mandates using a conservative margin of safety if ozone is employed as a fish disease control agent, because levels of 10–50 μg/L with contact times of 0.5–30 min are required to destroy the common viral and bacterial fish pathogens (Wedemeyer and Nelson 1977; Wedemeyer et al. 1978).

To help determine the cause of death and provide a background for future forensic diagnoses, we monitored physiological damage occurring during the acute exposures. Typical pathological changes in gill tissue caused by acute ozone exposure at 7 and 29 μg/L are shown in Fig. 3 (A–D). At the lowest ozone level (2 μg/L, not illustrated), there were no noticeable changes even after 96-h exposures. At 7 μg/L, hyperplasia of the lamellar epithelium was extensive by 96 h with some separation from the supporting elements (Fig. 3B). At the higher exposure level (29 μg/L) marked epithelial degeneration had occurred by 24 h and mortality was virtually complete within about 48 h. Survivors were moribund and showed very extensive cellular damage with severe hypertrophy, hyperplasia, epithelial degeneration, and necrosis (Fig. 3D). Similar pathology is seen following exposure to disinfectants such as chlorine and formalin and these changes are thus unfortunately not pathognomonic (Wedemeyer 1971). Thus, the potential for severe gill damage is also an obvious limiting factor in the use of ozone for fish disease control, and as much care will have to be

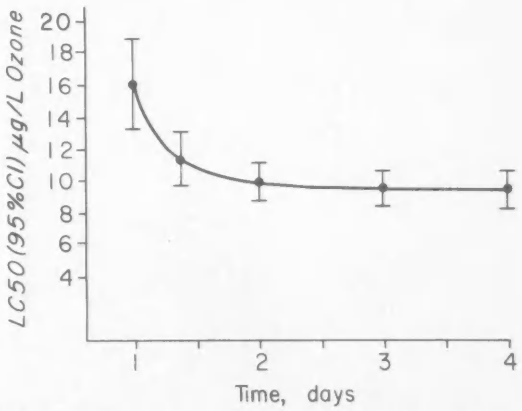
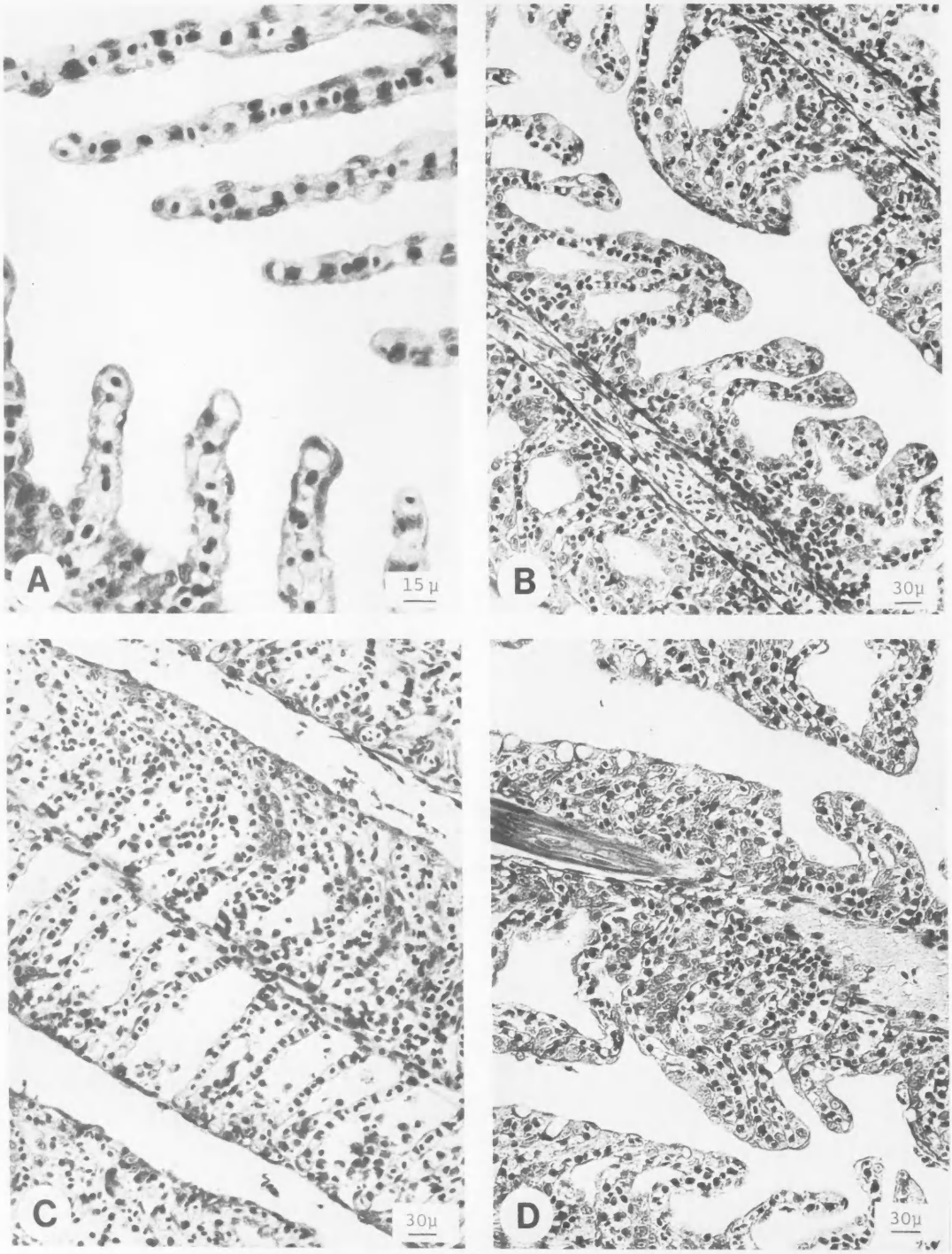


FIG. 2. Acute ozone toxocity curve for 10–13-cm rainbow trout in soft water at 10°C. The threshold toxicity is ~8 μg/L; the 96-h LC50 and 95% confidence interval is 9.3 (8.1–10.6) μg/L.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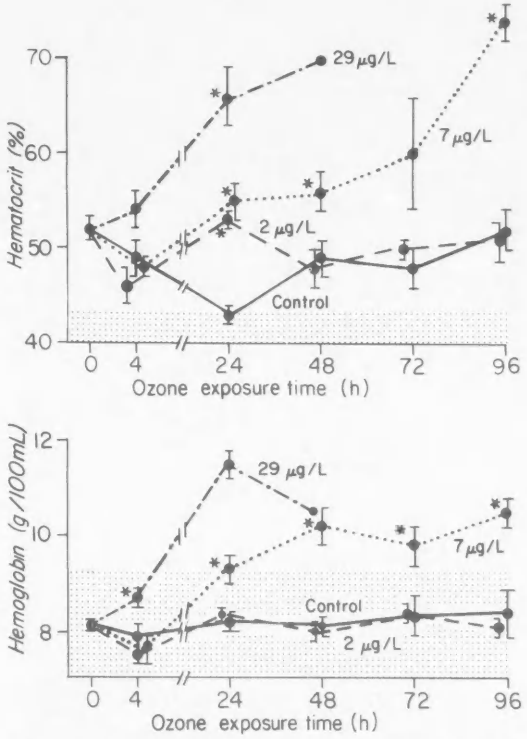

FIG. 4. Hematological dyscrasias occurring during acute ozone exposure; 10–13-cm rainbow trout in soft water at 10°C. Data given as $\bar{x} \pm$ SD, 10 fish per group; *denotes significant differences from initial values ($P = 0.05$). Stipled area shows approximate normal range limits.

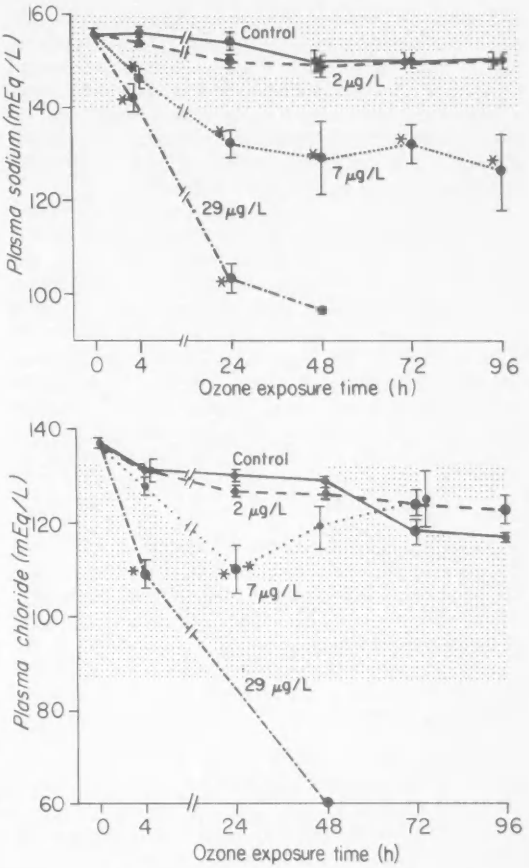

FIG. 5. Blood electrolyte disturbances occurring during acute ozone exposure at 5 µg/L in 10°C soft water are indicated by differential cell counts per 1000 cells ($\bar{x} \pm$ SD) 10 fish per group. Stipled area shows approximate normal range limits. *Indicates significantly different from 0-mo values ($P = 0.05$).

exercised in its application as is the case with the other disinfectants in common use.

To help clarify the degree to which the progressively severe gill pathological changes became physiologically limiting, we determined sequential blood chemistry profiles as a function of dose and contact time. Typical results are shown in Fig. 4 and 5. The stipled areas show the approximate limits of the normal range (Wedemeyer and Yasutake 1977). Hemoglobin and hematocrit levels increased significantly ($P = 0.05$) and remained elevated at ozone exposures of 7 µg/L and above suggesting reduced oxygen uptake due to gill damage, and severe hemoconcentration, since frank polycythemia would be unlikely to develop in such a short time period (Fig. 4). Peripheral blood smears revealed generally increased numbers of immature erythrocytes, indicating an increase in hematopoiesis, but no other dyscrasias. The fact that ozone has been shown to impair erythrocyte oxygen binding and transport capacity in humans may be relevant here, but it was not tested (Kindya and Chan 1976).

Ionoregulation was evidently compromised at the higher exposure levels, as indicated by the persistant plasma electrolyte imbalances that occurred (Fig. 5A, B). A serious hyponatremia and hypochloremia developed within 4 h at 29 µg/L O_3 which reached potentially lethal limits within 24–48 h. On the basis of case his-

FIG. 3. Typical effects of acute ozone exposure on rainbow trout gill lamellae. Hematoxylin and eosin stain. (A) Unexposed control. (B) 7 µg/L, 96-h exposure; hyperplasia of the lamellar epithelium gives a "clubbing" appearance. (C) 29 µg/L, 24-h exposure; much of the lamellar epithelium appears to be gone. (D) 29 µg/L, 24-h exposure; extensive alteration of the normal architecture of the lamellae is evident with severe epithelial hyperplasia and fushion of the lamellae.

tory data, we consider declines in plasma chloride and sodium to levels below ~100 meq/L to be life-threatening for salmonids. At the 7 μg/L O_3 exposure level, a significant ($P = 0.05$) though biologically mild hyponatremia and hypochloremia developed, which was correlated with the gill histological changes. The 2 μg/L O_3 acute exposure level caused no blood electrolyte imbalances or gill histological changes.

That ozone exposure also causes a severe, generalized, stress response was illustrated by the rapid, intense hyperglycemia that developed (Fig. 6). Again, the stipled area shows the approximate normal range limits. Blood sugar levels were abnormal within 4 h at the 29 μg/L O_3 levels, and within 24 h mortality was essentially complete. At 7 μg/L O_3, hyperglycemia was severe by 24 h and potentially lethal by 96 h. At the 2 μg/L ozone level, blood glucose values remained essentially within the control range although they increased somewhat after the 1st d of exposure.

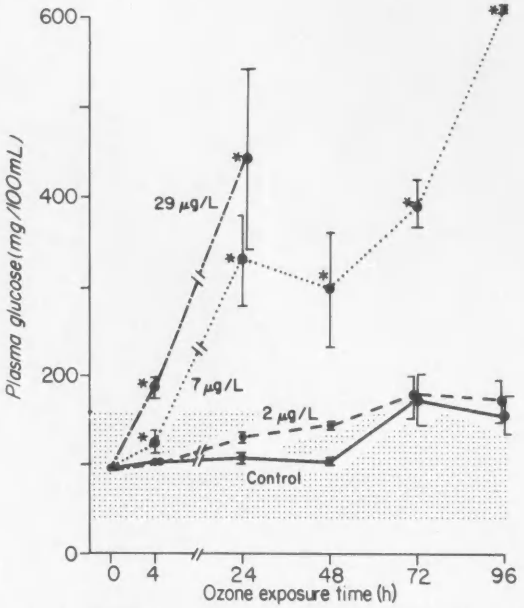

FIG. 6. Severe hyperglycemia resulting from the stress of acute ozone exposure; 10–13 cm rainbow trout, soft water, 10°C. Data given as $\bar{x} \pm$ SD, 10 fish per group; *indicates significant difference from initial values ($P = 0.05$). Stipled area shows estimated normal range limits.

CHRONIC TOXICITY

Due to the thermodynamic instability of ozone and the unavailability of feedback control systems, it was not possible to achieve completely stable, graduated, ozone exposure levels over long time periods and only partial chronic (3-mo) tests could be carried out. An average ozone concentration ($\bar{x} \pm$ SD) of 2.3 ± 0.2 μg/L was achieved in the first 3-mo test and 5.0 ± 0.4 μg/L in the second.

TABLE 1. Summary of hematological changes in 10–13-cm rainbow trout caused by 3-mo chronic ozone exposure at 2 μg/L in 10°C soft water. Values are for means of differential cell counts per 1000 cells ($\bar{x} \pm$ SD) 10 fish per group. *Significant within group difference from initial values ($P = 0.05$).

Blood cell type and exposure group	Value after exposure (mo)			
	0	1	2	3
Erythrocytes, mature				
control	955±14	930±21*	942±18	944±20
test	955±14	944±25	954±38	927±33*
Erythrocytes, immature				
control	31±13	44±18	16±13*	23±17
test	31±13	29±14	11±6*	20±16
Lymphocytes				
control	13±7	26±10*	27±14*	32±23*
test	13±7	25±12*	31±31*	46±31*
Thrombocytes				
control	0.2±0.4	0.1±0.3	1.5±3.2	1.9±3.2
test	0.2±0.4	3.6±3.0*	12.0±13.3*	8.2±6.2*
Neutrophils, mature				
control	0.2±0.4	0	0.1±0.3	0
test	0.2±0.4	0	0.2±0.7	0.1±0.2
Neutrophils, immature				
control	0.2±0.4	0	0	0
test	0.2±0.4	0	0.1±0.2	0

TABLE 2. Summary of blood chemistry changes in 10–13-cm rainbow trout caused by 3-mo chronic ozone exposure at 2 μg/L in 10°C soft water. Values are given as means ($\bar{x}$ ± SD) 20 fish per group. None of the test groups was significantly altered from its corresponding control group ($P = 0.05$). *Significant within group difference from initial values ($P = 0.05$).

Physiological parameter and exposure group	Value after exposure (mo)			
	0	1	2	3
Plasma chloride (meq/L)				
control	134±6	130±3	133±3	132±2
test	134±6	129±3	130±4	130±4
Hematocrit (%)				
control	45±7	40±3	34±5*	33±3*
test	45±7	43±5	36±7*	36±6*
Hemoglobin (g/100 mL)				
control	8.9±0.7	7.6±0.6*	6.6±1.2*	6.1±1.0*
test	8.9±0.7	7.8±0.7*	6.7±1.1*	6.6±1.0*
Growth (fish wt, g)				
control	29±9	28±8	21±10*	19±6*
test	29±9	24±8	22±9*	21±10*
Plasma glucose (mg/100 mL)				
control	131±22	67±6*	72±5*	66±13*
test	131±22	68±8*	74±10*	75±11*
Plasma sodium (meq/L)				
control	144±5	150±4	151±3	145±5
test	144±5	149±4	146±3	151±4

At the 2 μg/L chronic exposure level, there were no mortalities in either the test or control groups and the only physiological change that could be associated with ozone was a mild thrombocytosis in the test fish (Table 1). There was a tendency toward lymphocytosis and a normochronic, nomocytic anemia in both the control and test fish, as indicated by the differential blood cell counts and the gradual decline in hemoglobin and hematocrit (Table 1, 2). A mild but persistent hypoglycemia was also evident. Since these abnormalities were also seen in the control groups, they were undoubtedly the result of inanition caused by the minimal feeding regimen required to control fluctuations in ozone demand and help stabilize exposure levels. This was substantiated by the approximately equal weight loss in both the experimental and control groups (Table 2). The gill histological changes that occurred at the 2 μg/L exposure level were minor (Fig. 7B, C) and apparently did not compromise functions such as ionoregulation — as indicated by the fact that stable plasma Na^+ and Cl^- levels were maintained (Table 2).

At the 5 μg/L chronic exposure level, experience revealed that the feeding level could be increased enough to maintain a moderate weight gain before an excessively fluctuating ozone demand was created. Growth was thus normal in the controls (Table 3). However, although food was available, feeding behavior in the ozone-exposed groups began to decrease noticeably after ~2 mo and a significant ($P = 0.05$) within-group weight loss, together with a mild hypoglycemia, occurred during the 3rd mo. A mild polycythemia with falling immature erythrocyte counts and lymphocytopenia were also significant ($P = 0.05$) within-group trends (Table 3, 4). These were presumably physiological compensations reflecting impaired gas exchange due to the gradually developing gill hypertrophy.

Histologically, the gill lamellae in the 5 μg/L test groups exhibited varying degrees of epithelial damage (Fig. 7D). Hypertrophy was more extensive but generally was qualitatively similar to that occurring at the 2 μg/L ozone exposure level. The one exception was that after 3 mo, hypertrophy of occasional individual epithelial cells in the 5 μg/L test groups resulted in an irregular-appearing lamellar surface. This was not seen at the lower exposure level (Fig. 7). Again, the degree of gill damage that occurred did not compromise ionoregulation, as indicated by the stable plasma Na^+, Cl^- levels that were maintained (Table 3). In general, as was true of the acute ozone toxicity results, the gill pathological changes were not pathognomonic, but typical of those seen following exposure to agents such as chlorine, ammonia, and formalin (Wedemeyer 1971; Smart 1976).

In summary, the acute toxicity of ozone is high. The 96-h LC50 and 95% C.I. was 9.3 (8.1–10.6) μg/L O_3 for 10–13-cm rainbow trout. Death following acute exposures is most likely due to severe gill lamellar epithelial tissue destruction accompanied by massive hydromineral imbalances.

Partial chronic testing revealed that ozone exposure

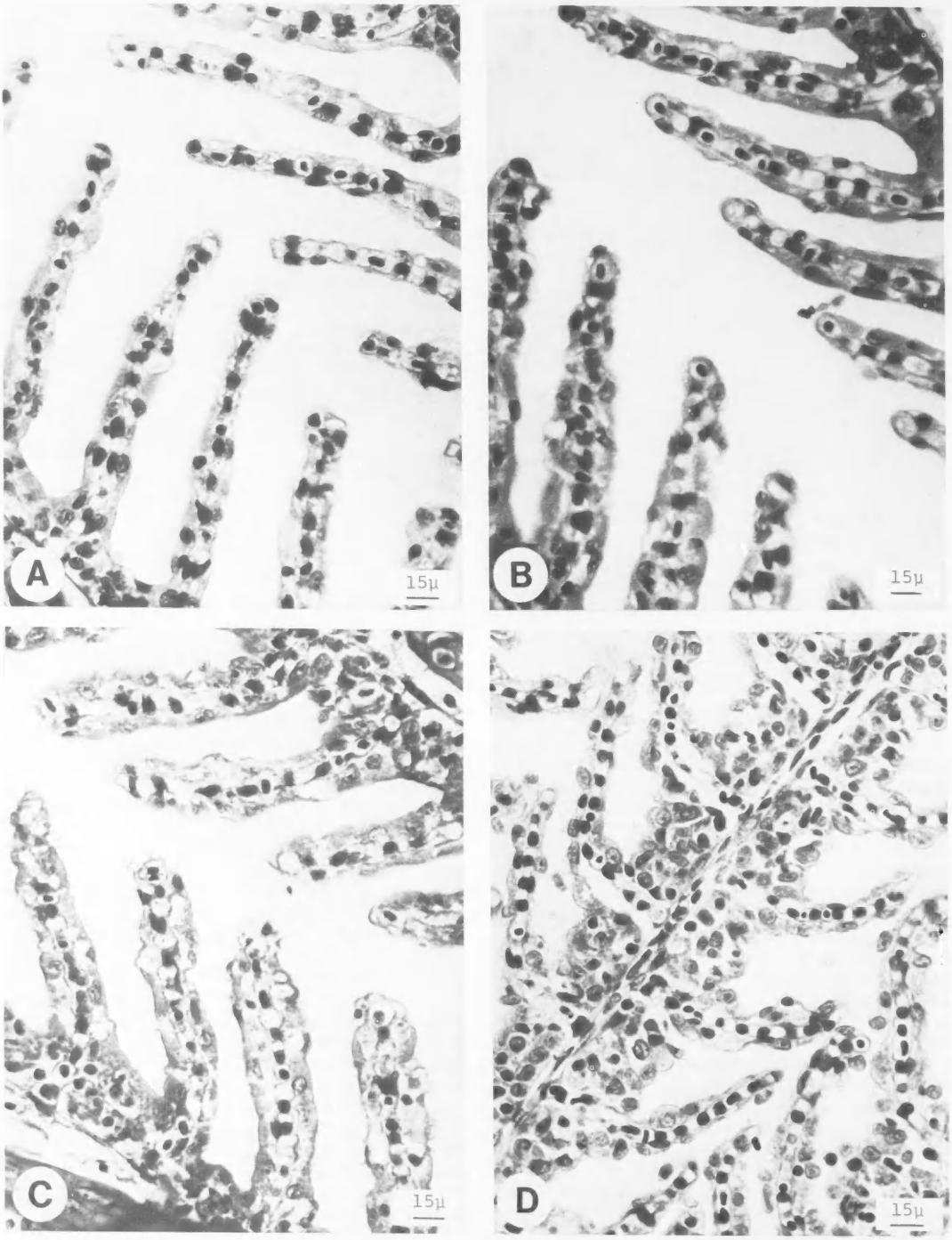
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TABLE 3. Summary of physiological changes in 10–13-cm rainbow trout caused by 3-mo chronic ozone exposure at 5 μg/L in 10°C soft water. Values given as means ($\bar{x} \pm$ SD) 20 fish per group. *Significant within group difference from initial values ($P = 0.05$).

Physiological parameter and exposure group	Value after exposure (mo)			
	0	1	2	3
Plasma chloride (meq/L)				
control	133±4	134±3	130±3	121±4*
test	133±4	134±2	130±5	134±4
Hematocrit (%)				
control	47±3	42±3	54±3*	38±3*
test	47±3	45±4	50±4*	45±8
Hemoglobin (g/dL)				
control	7.6±0.6	7.6±0.6	7.3±0.3	7.0±0.6*
test	7.6±0.6	7.4±0.8	8.1±0.5	7.4±0.7
Growth (fish wt, g)				
control	20±5	25±4*	38±10*	42±5*
test	20±5	25±7	35±12*	28±11*
Plasma glucose (mg/dL)				
control	92±11	90±6	105±21	90±16
test	92±11	90±8	93±11	81±18*
Plasma sodium (meq/L)				
control	159±2	153±3	157±4	143±5*
test	159±2	154±3	152±3	152±8

TABLE 4. Summary of hematological changes in 10–13-cm rainbow trout caused by 3-mo chronic ozone exposure at 5 μg/L in 10°C soft water. Values are for means of differential cell counts per 1000 cells ($\bar{x} \pm$ SD) 10 fish per group. *Significant within group difference from initial values ($P = 0.05$).

Blood cell type and exposure group	Value after exposure (mo)			
	0	1	2	3
Erythrocytes, mature				
control	922±31	924±22	864±33	921±27
test	922±31	942±22	941±24*	957±19*
Erythrocytes, immature				
control	42±16	22±8*	30±17	26±10*
test	42±16	12±8*	13±4*	9±6*
Lymphocytes				
control	44±23	43±14	74±18*	42±24
test	44±23	40±23	40±18	28±11*
Thrombocytes				
control	10.7±10.3	10.8±11	30.7±18.6*	10.0±10.0
test	10.7±10.3	5.6±4.5	6.6±7.3	6.0±6.6
Neutrophils, mature				
control	0.3±0.7	0.1±0.3	0.5±0.8	0.5±0.6
test	0.3±0.7	0.1±0.3	0.1±0.3	0.1±0.3
Neutrophils, immature				
control	1.4±2.3	0.5±1.0	0.7±0.8	0.2±0.5
test	1.4±2.3	0	0.2±0.6	0.1±0.3

FIG. 7. Typical gill histological changes following chronic ozone exposure. (A) Unexposed control. (B) 2 μg/L, 4-wk exposure. (C) 2 μg/L, 12-wk exposure. (D) 5 μg/L, 12-wk exposure. Gill lamellae show varying but minimal epithelial hypertrophy.

at 2 μg/L for 3 mo caused only minimal physiological changes, and a provisional maximum safe exposure level of 2 μg/L is suggested for salmonids, pending completion of life cycle studies and development of an analytic method specific for ozone.

In terms of environmental impact, if ozone is used as a replacement for chlorine in industrial applications such as sewage treatment or power plant cooling systems, it should be removed or diluted to about the 2 μg/L level before it is allowed to come in contact with salmonids in receiving waters.

Acknowledgments

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AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1975. Standard methods for the examination of water and wastewater. 14th ed., Washington, D.C. 874 p.

ARTHUR, J. R., V. MATTSON, D. OLSON, G. GLASS, B. HALLIGAN, AND C. WALBRIDGE. 1975. Comparative toxicity of sewage — effluent disinfection to freshwater aquatic life. Ecol. Res. Ser. DPE-600/3-75-012, U.S. Environ. Prot. Agency, Environ. Res. Lab., Duluth, Minn.

BENOIT, R. F., AND N. A. MATLIN. 1966. Control of *Saprolegnia* on eggs of rainbow trout (*Salmo gairdneri*) with ozone. Trans. Am. Fish. Soc. 95: 430–432.

BLOGOSLAWSKI, W., L. FARRELL, R. GARCEAU, AND P. DERRIG. 1976. Production of oxidants in ozonized seawater. Second Int. Symp. Ozone Technol. Ozone Press Int., Jamesville, N.Y. 725 p.

BLOGOSLAWSKI, W., AND M. E. STEWART. 1977. Marine applications of ozone water treatment, p. 266–276. *In* E. G. Fochtam et al. [ed.] Forum on ozone disinfection. Int. Ozone Inst., Syracuse, N.Y.

CARLSON, R. M., AND R. CAPLE. 1977. Chemical, biological implications of using chlorine and ozone for disinfection. Ecol. Res. Ser. EPA-600/3-77-066. Environ. Res. Lab., U.S. Environ. Prot. Agency, Duluth, Minn.

CONRAD, J. F., R. A. HOLT, AND T. D. KREPS. 1975. Ozone disinfection of flowing water. Prog. Fish-Cult. 37: 134–136.

EPA (ENVIRONMENTAL PROTECTION AGENCY). 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Ecol. Res. Ser. EPA-660/3-75-099, U.S. Environ. Prot. Agency, Natl. Environ. Res. Cent., Corvallis, Oreg.

HAMILTON, M. A., R. C. RUSSO, AND R. V. THURSTON. 1977. The trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11: 714–719.

HARRIS, W. C. 1974. Ozone disinfection. J. Am. Water Works Assoc. 64: 182–183.

HONN, K. V., AND W. CHAVIN. 1976. Utility of ozone treatment in the maintenance of water quality in a closed marine system. Mar. Biol. 34: 210–219.

HUBBS, C. L. 1930. The high toxicity of nascent oxygen. Physiol. Zool. 3: 441–460.

KINDYA, R. J., AND P. C. CHAN. 1976. Effect of ozone on erythrocyte membrane adenosinetriphosphate. Biochem. Biophys. Acta 429: 608–615.

MCLEAY, D. 1975. Sensitivity of blood cell counts in juvenile coho salmon (*Oncorhynchus kisutch*) to stressors including sublethal concentrations of pulp mill effluent and zinc. J. Fish. Res. Board Can. 32: 2357–2364.

NEBEL, C., R. D. GOTTSCHLING, R. L. HUTCHINSON, T. J. MCBRIDE, D. M. TAYLOR, J. L. PAVONI, M. E. TITTLEBAUM, H. E. SPENCER, AND M. FLEISHMAN. 1973. Ozone disinfection of industrial-municipal secondary effluents. J. Water Pollut. Control Fed. 45: 2493–2507.

ROSENLUND, B. 1975. Disinfection of hatchery influent by ozonation and the effects of ozonated water on rainbow trout, p. 59–69. *In* W. Blogoslawski and R. Rice [ed.] Aquatic Applications of Ozone, Int. Ozone Inst., Cleveland, Ohio. 226 p.

SIEGRIST, H. W., D. G. TUTTLE, AND S. B. MAJUMDAR. 1975. Technical and economic considerations of biocide system options for cooling water systems: a review. Proc. 2nd Int. Symp. Ozone Tech. Ozone Press Int., Jamesville, N.Y. 725 p.

SMART, J. 1976. The effect of ammonia exposure on gill structure of the rainbow trout (*Salmo gairdneri*). J. Fish Biol. 8: 471–475.

WEDEMEYER, G. 1971. The stress of formalin treatments in rainbow trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 28: 1899–1904.

WEDEMEYER, G., AND N. C. NELSON. 1977. Survival of two bacterial fish pathogens (*Aeromonas salmonicida* and the enteric redmouth bacterium) in ozonated, chlorinated, and untreated waters. J. Fish. Res. Board Can. 34: 429–432.

WEDEMEYER, G., N. C. NELSON, AND C. A. SMITH. 1978. Survival of the salmonid viruses infectious hematopoeitic necrosis (IHNV) and infectious pancreatic necrosis (IPNV) in ozonated, chlorinated and untreated waters. J. Fish. Res. Board Can. 35: 875–879.

WEDEMEYER, G., AND W. T. YASUTAKE. 1977. Methods for the clinical assessment of the effects of environmental stress on fish health. U.S. Fish Wildl. Serv. Tech. Pap. 89: 18 p.

Physiology of Salinity-Dependent Naphthalene Toxicity in *Fundulus heteroclitus*[1]

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The influence of salinity on mortality in mummichogs, *Fundulus heteroclitus*, subjected to naphthalene and the physiological bases for the salinity-dependent mortalities were determined. Fish were acclimated to five salinities, 2, 8, 15, 23, and 33‰, and exposed to 6 and 4 mg naphthalene/L. At 4 mg/L, fish survival was greater at hypo- and near isoosmotic (15‰) salinities than at hyperosmotic salinities. At 6 mg/L, survival was generally greatest at 8 and 15‰ and decreased at the salinity extremes. In fish exposed to 4 mg naphthalene/L, uptake was more rapid at the iso- and hyperosmotic salinities than at hypoosmotic salinities and osmotic imbalance occurred only at hyperosmotic salinities. Serum cortisol levels and oxygen consumption rates increased at all salinities upon exposure to 4 mg naphthalene/L. However, the most significant increases in oxygen consumption rates occurred at hyperosmotic salinities. Thus, the greater naphthalene uptake, osmotic imbalance, and metabolic stress occurring at hyperosmotic salinities may contribute to the increased mortality of naphthalene-exposed fish acclimated to hyperosmotic salinities.

Key words: toxicity, naphthalene, petroleum hydrocarbon, teleost, *Fundulus heteroclitus*, salinity, osmoregulation, uptake, oxygen consumption, stress

LEVITAN, W. M., AND M. H. TAYLOR. 1979. Physiology of salinity-dependent naphthalene toxicity in *Fundulus heteroclitus*. J. Fish. Res. Board Can. 36: 615–620.

Nous avons déterminé l'influence de la salinité sur la mortalité de choquemorts, *Fundulus heteroclitus*, exposés au naphthalène, ainsi que les bases physiologiques des mortalités liées à la salinité. Les poissons ont été acclimatés à cinq salinités, 2, 8, 15, 23 et 33‰, et exposés à 6 et 4 mg de naphthalène/L. A 4 mg/L, la survie des poissons est meilleure aux salinités hypo- et presque isotoniques (15‰) qu'aux salinités hypertoniques. A 6 mg/L, la survie est généralement maximale à 8 et 15‰ et diminue aux extrêmes de salinité. Chez les poissons exposés à 4 mg de naphthalène/L, l'absorption est plus rapide aux salinités iso- et hypertoniques qu'aux salinités hypotoniques et le déséquilibre osmotique ne se produit qu'aux salinités hypertoniques. Les niveaux de cortisol sérique et les taux de consommation d'oxygène augmentent à toutes les salinités après exposition à 4 mg de naphthalène/L. Cependant, les plus importantes augmentations des taux de consommation d'oxygène se produisent aux salinités hypertoniques. Il se peut donc que la plus grande absorption de naphthalène, le déséquilibre osmotique et le stress métabolique qui se produisent aux salinités hypertoniques contribuent à des mortalités accrues chez des poissons exposés au naphthalène et acclimatés à des salinités hypertoniques.

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DAILY and seasonal fluctuations in estuarine environmental conditions, such as salinity, have necessitated the consideration of environmental variables in toxicity studies of estuarine organisms. Previous studies have indicated that salinity can affect the survival of euryhaline fish subjected to such perturbations as synthetic detergents (Eisler 1965), crude oils (Eisler and Kissil 1975) and DDT (Leadem et al. 1974). Generally, fish at hypoosmotic and especially isoosmotic salinities are better able to survive the perturbations than those at hyperosmotic salinities. Although pollutant–salinity interactions in fish have been investigated with regard to osmotic constituents (Eisler and Edmunds 1966; Kinter et al. 1972; Leadem et al. 1974; Morrow et al. 1975) and metabolic stress (Kloth and Wohlschlag

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1972), little attempt has been made to relate physiological function to salinity-dependent mortality trends.

Petroleum aromatic hydrocarbons, including naphthalene and its derivatives, have been found to be among the most toxic compounds in oil. The early literature on exposure of teleosts to naphthalene, as reviewed by Boylan and Tripp (1971), placed acute lethality in the range of 2–5 mg naphthalene/L. More recently, Anderson et al. (1974) reported a 24-h LC50 of 2.4 mg naphthalene/L for the sheepshead minnow (*Cyprinodon variegatus*) and DiMichele and Taylor (1978) found that 8 mg naphthalene/L was lethal to 100% of *Fundulus heteroclitus* exposed for 4 d.

The purpose of this study was to correlate toxicity of naphthalene with osmotic and metabolic effects of pollutant–salinity interactions in a euryhaline fish. Mummichogs, *F. heteroclitus*, were subjected to naphthalene at various salinities to define salinity-dependent mortality trends. Tissue naphthalene uptake, serum osmotic constituents, serum cortisol, and oxygen consumption were measured in similarly exposed fish to identify the physiological bases for the observed mortality patterns.

Materials and Methods

Fundulus heteroclitus, averaging 7.4 cm total length and 5.2 g weight, were collected from Canary Creek Marsh, Lewes, Delaware. The fish were acclimated for 1 mo in 76-L aquaria containing artificial seawater (Instant Ocean) at salinities of 2, 8, 15, 23, and 33‰. The isoosmotic point of *F. heteroclitus* serum is 14‰ (Pickford et al. 1969).

Each experimental aquarium was equipped with an undergravel filter and housed 15–20 fish (female:male ratio was 1.1:1) in 57 L of water. The experiments were run in winter and early spring, before full gonadal development had occurred. Experimental aquaria were maintained on an LD photoperiod of 14:10. Fish were fed Purina Trout Chow (Ralston-Purina Corp.) ad lib. until 36 h before each experiment. They were fasted from that time until the end of the experiment.

Bioassays were run at 6 mg naphthalene/L (16 and 20°C) and 4 mg naphthalene/L (21°C) and the physiological tests were carried out at 4 mg naphthalene/L (20–21°C). These concentrations were selected to provide a model system for laboratory analysis of the physiological processes involved in acute naphthalene toxicity. In all experiments, naphthalene was introduced into the tanks via an ethanol stock solution. With the exception of the uptake experiment, all experiments were run with ethanol controls. Test naphthalene concentrations were kept constant by hourly additions of stock solution. Amounts to be added were calculated from regular monitoring of the naphthalene concentrations in the tanks.

Naphthalene concentrations in tank water and whole fish tissues were measured using modifications of the methods described by Neff and Anderson (1975). For tank water, samples were diluted to 4 mL with distilled water. The sample size was determined by the dilution factor necessary to bring the concentration into the sensitive range of the standard curve. The samples were acidified with 0.1 mL concentrated HCl and extracted with 4 mL of spectrophotometric grade hexane. The naphthalene concentrations were read at 221 nm. Recovery of added naphthalene was determined to be 100.52 ± 2.49% (SE).

For tissue naphthalene determinations, fish were blotted and homogenized over ice for 1 min with 20 mL of specgrade hexane in a Waring blender. After centrifugation, the supernatants were transferred to two 7.4-mL vials containing 2 g of activated Florisil. Homogenates were stored 24 h in a cool, dark place with occasional shaking. An aliquot of the hexane extract was then diluted to 4 mL with specgrade hexane and read at 221 nm. Recovery of naphthalene from tissue was 91.5 ± 3.25%.

Blood was collected from the caudal artery in unheparinized capillary tubes after severing the caudal peduncle. Serum osmolality (mosmol/kg) was determined with a vapor pressure osmometer (Wescor Model 5100) and serum sodium and potassium (mmol/L) were analyzed on a flame photometer. Serum cortisol was measured using a radioimmunoassay technique described by the antibody supplier (Endocrine Sciences, Tarzana, Calif.) with modifications reported by Leach and Taylor (1977).

To measure oxygen consumption rates, we transferred fish from acclimation tanks into 1-L flasks (one fish per flask) containing 1 L of the appropriate salinity acclimation water. The flasks were covered with translucent plastic and left undisturbed the 1st h after transfer. At the end of that time naphthalene and ethanol were added and the flasks were left covered for another hour. They were then made airtight and at 0, 20, 40, and 60 min, water samples were removed and measured for dissolved oxygen using a microadaptation of the Winkler–Azide method (APHA 1971). Oxygen consumption rates (mg $O_2 \cdot g$ wet $wt^{-1} \cdot h^{-1}$) were calculated from the dissolved oxygen measurements.

Duncan's multiple range test was used to compare the effects of salinity treatment. Student's *t*-test was used to compare the means of naphthalene-exposed and ethanol control fish at each test salinity.

Results

In the bioassay experiments, no mortalities were found in the ethanol control tanks nor were the mortalities in the experimental tanks dependent on size or sex. At an exposure of 4 mg naphthalene/L at 21°C (Fig. 1), fish acclimated to hypoosmotic (2 and 8‰) and isoosmotic (15‰) salinities had a significantly higher survival rate than did fish acclimated to hyperosmotic (23 and 33‰) salinities. The influence of salinity on survival was significant at $P < 0.001$. At 6 mg naphthalene/L and 16°C (Fig. 1), survival was greatest at 8 and 15‰, intermediate at 2‰, and lowest at 23 and 33‰ ($P < 0.05$). Survival of fish exposed to 6 mg naphthalene/L and 20°C (Fig. 1) was greatest at 8‰ and decreased at the salinity extremes ($P < 0.01$).

Serum osmolalities in fish exposed to 4 mg naphthalene/L for 12 h at 2 and 8‰ were similar to the levels in control fish (Fig. 2). At 15‰, there was a small but significant ($P < 0.05$) elevation in the serum osmolality of exposed fish at 8 and 12 h. At 23‰, osmolality in exposed fish was elevated ($P < 0.05$) at 2 h but declined to control levels by 12 h. At 33‰, osmolality increased 30% in 4 h to 431.1 mosmol/kg

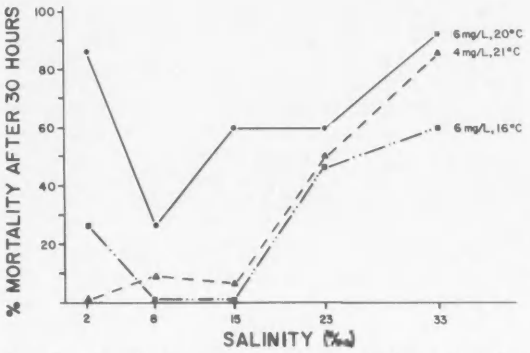

FIG. 1. Cumulative percent mortality at 30 h as a function of salinity in *F. heteroclitus* exposed to naphthalene at 4 or 6 mg/L ($n = 15$).

($P < 0.05$). Again, control levels were achieved by 12 h. Sodium and potassium concentrations paralleled osmolality and are not included here.

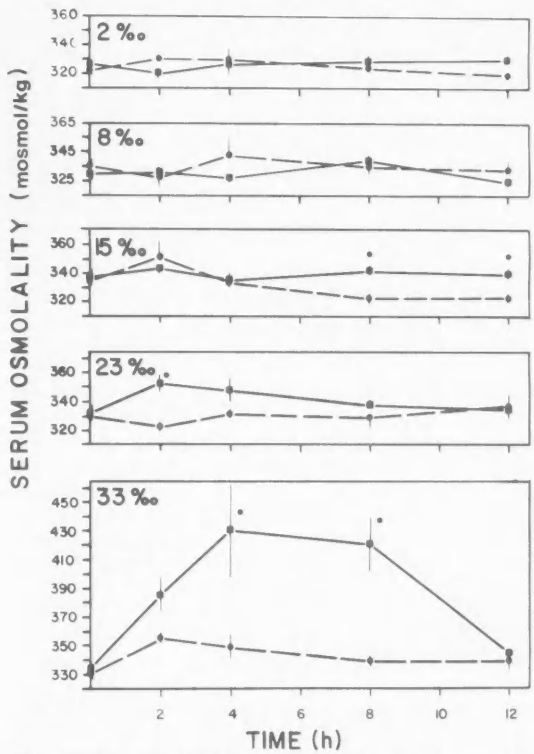

FIG. 2. Mean (±1 SE) serum osmolality in *F. heteroclitus* exposed to 4 mg naphthalene/L at five salinities. Solid lines are experimental fish and broken lines are corresponding control groups ($n = 4$). An asterisk indicates significant difference ($P < 0.05$) between treated and control means.

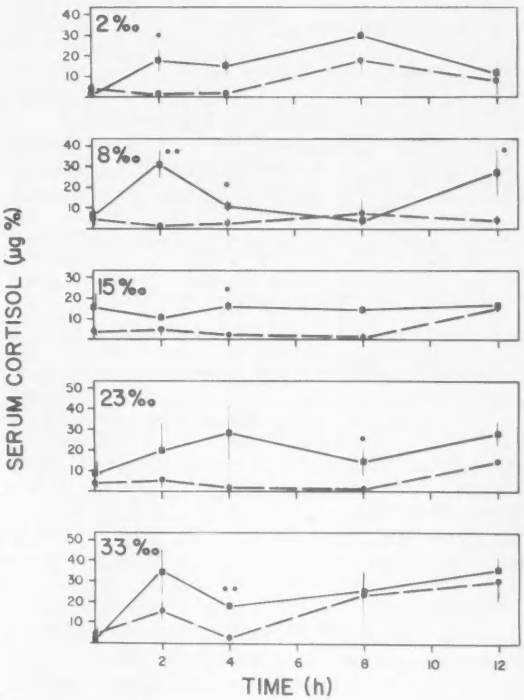

FIG. 3. Mean (±1 SE) serum cortisol at the five test salinities. Solid lines are experimental fish (4 mg naphthalene/L) and broken lines the corresponding control groups ($n = 4$). Asterisks indicate significant difference between treated and control means: * = $P < 0.05$, ** = $P < 0.01$.

Upon addition of naphthalene, cortisol levels generally increased independently of salinity (Fig. 3). At 2 and 4 h, cortisol levels in exposed fish were greater than in control fish, although the differences were not statistically significant in every case. Control cortisol levels were elevated by 12 h in all but the 8‰ group.

Mean hematocrits were 35–55% with no consistent differences between salinity groups or between naphthalene-exposed and ethanol-control fish.

Naphthalene uptake was rapid in the first 4 h of exposure, and accumulation was still occurring at 12 h (Fig. 4). During the initial period, fish acclimated to 2, 8, 15, and 23‰ had similar tissue naphthalene accumulations but fish at 33‰ had significantly higher concentrations ($P < 0.05$ at 1 h; $P < 0.01$ at 2 and 4 h). At 8 and 12 h, two groupings became apparent: fish acclimated to higher salinities (15, 23, and 33‰) had higher tissue concentrations than those acclimated to 2 and 8‰. After 12 h, tissue naphthalene concentrations in all groups were 3–6 times the ambient concentration.

Oxygen consumption rates of fish exposed to 4 mg naphthalene/L were significantly higher ($P < 0.05$) than those of control fish at all salinities except 8‰

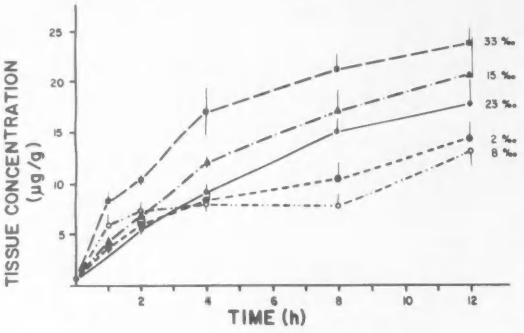

FIG. 4. Mean (±1 SE) uptake of naphthalene by *F. heteroclitus* exposed to 4 mg naphthalene/L at five salinities ($n = 6$).

(Fig. 5). In ethanol-control fish, rates did not vary significantly with salinity; but in naphthalene-exposed fish, oxygen uptake rates were elevated to a greater extent at increased salinities ($P < 0.05$; 2 vs. 8–33‰, 8–15 vs. 23–33‰).

Discussion

These experiments indicate that a salinity-dependent mortality trend exists in the euryhaline fish *Fundulus heteroclitus* exposed to naphthalene. Mortality was greatest at hyperosmotic salinities in the three naphthalene dosage–temperature combinations tested. Fish exposed to naphthalene under hypoosmotic conditions (2‰) showed a marked increase in mortality when the higher of the two tested doses was applied at 20°C but not at 16°C. The mortality trend was found to correlate with and probably resulted from the interaction of a combination of physiological factors including osmoregulatory dysfunction, naphthalene uptake, and metabolic stress.

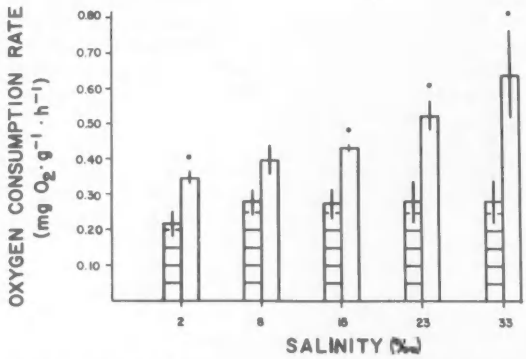

FIG. 5. Mean (±1 SE) oxygen consumption as a function of salinity in 4 mg naphthalene/L exposed fish (plain bar) and ethanol controls (hatched bar). Bars identified by asterisks are significantly different than controls at $P < 0.05$ ($n = 5$).

Salinity-dependent mortality has been reported for fish exposed to other toxins. For example, Eisler (1965) found that survival in *F. heteroclitus* and juvenile eels (*Anguilla rostrata*) exposed to 96-h LC50 concentrations of synthetic detergents was greatest in brackish water, least in seawater, and intermediate in freshwater. Leadem et al. (1974) found that rainbow trout (*Salmo gairdneri*) could not be maintained on 1-mg oral doses of DDT in 100% seawater but survived in freshwater and 33% seawater.

Because serum osmotic components were temporarily elevated at hyperosmotic salinities upon exposure to 4 mg naphthalene/L, it is likely that osmoregulatory dysfunction may have contributed to the observed salinity-dependent mortality. Elevated serum osmotic components in fish at hyperosmotic salinities may be a general effect of organic toxins as the effect has been found with both chlorinated hydrocarbons (Eisler and Edmunds 1966; Leadem et al. 1974; Kinter et al. 1972) and petroleum aromatic hydrocarbons (Morrow et al. 1975; and this study).

It is possible that naphthalene, a lipophilic substance, may inhibit Na, K ATPase and thereby permit an increase in serum osmolality at hyperosmotic salinities. This was proposed by Kinter et al. (1972) on the basis of their finding that seawater-adapted *Anguilla rostrata* exposed to DDT had a 30% higher osmolarity than ethanol-control fish and a 38% inhibition of gill Na, K ATPase. Also, seawater-adapted *F. heteroclitus* exhibited increased osmolarity upon exposure to both DDT and PCBs; the latter having previously been shown to inhibit Na, K ATPase.

The observed salinity-dependent uptake of naphthalene in *Fundulus* is an obvious potential contributor to salinity-dependent mortality. Two possible sources of increased naphthalene uptake at high salinities are (1) osmoregulatory mechanisms, and (2) ventilation rates. In euryhaline fish there is generally a direct correlation between increased external salinity, increased drinking rate, and increased flow of sodium across the intestinal wall (Potts and Evans 1967). As a result, more naphthalene may come into contact with transport epithelia at hyperosmotic than isoosmotic salinities, leading to increased uptake. Ventilation rates were not measured directly in our experiments, but increased oxygen consumption would be expected to be associated with increases in both ventilation volume and cardiac output (Shelton 1970). Thus, toxin uptake should be greater at higher salinities due to the increased diffusion at elevated water and blood flow rates.

The third probable contributor to the increased mortality at salinity extremes is metabolic stress. The two indicators used, serum cortisol and oxygen consumption, were both elevated by naphthalene exposure. The cortisol response was independent of salinity and less consistent than reported by DiMichele and Taylor (1978) for 15-d exposures to lower doses of naphthalene. In contrast, the increment in oxygen consumption

attributable to naphthalene exposure was directly proportional to salinity at salinities above 15‰. Oxygen consumption has previously been found to increase in fish exposed to petroleum hydrocarbons (Anderson et al. 1974; Thomas and Rice 1975; Neff et al. 1976; Rice et al. 1977).

Overall elevations in metabolic rate may have been caused by a generalized, nonspecific stress response to naphthalene exposure. The salinity-dependent effect may have resulted from various specific energy-requiring enzymatic processes. Sabo et al. (1975) and Burns and Sabo (1975) have reported that exposure of fish to petroleum hydrocarbons stimulates various metabolic and detoxifying processes. Because fish at hyperosmotic salinities had higher tissue naphthalene concentrations than fish acclimated to lower salinities, more tissue damage may have occurred resulting in increased activity of anabolic and detoxifying pathways. Increased energy demand for ion transport processes is suggested by the observed osmotic recovery at 12 h in hyperosmotic conditions. Finally, because blood stasis occurs in naphthalene-exposed *Fundulus* (DiMichele and Taylor 1978), oxygen is not delivered to the tissues efficiently. Thus increased respiratory compensation could result in increased oxygen consumption as well as greater energy utilization to support heightened ventilation rates.

Temperature may also affect the stress response; higher temperatures generally result in greater oxygen demand and metabolic stress. Metabolic effects presumably account for the increased mortality in fish exposed to 6 mg naphthalene/L at 20°C compared to those exposed at 16°C.

The salinity-dependent increase in oxygen consumption did not occur in control fish. Nordlie (1978) defined the lack of oxygen consumption change in response to salinity as a Type I response, associated with a minimal influence of salinity variation on osmoregulatory systems, and proposed that this response is characteristic of euryhaline teleosts. This classification is consistent with our observations and with the ambient salinity range of *F. heteroclitus*. Loss of the Type I response in naphthalene-exposed fish may, in part, result from increased energy demand on an already active osmoregulatory system.

In summary, it appears that osmoregulatory dysfunction and increased naphthalene uptake may occur in hyperosmotic acclimation as a result of toxin influences on ion transport systems. The increased energy demands of transport and detoxifying systems, as well as others not discussed here, would result in elevated metabolic rates at high salinities. Osmotic stress, increased toxin uptake, and increased energy demand probably all contribute to the observed salinity-dependent mortality trend. Due to the lipophilic nature of many toxic substances, the effects observed with naphthalene may apply to other pollutants as well.

The naphthalene doses used in this study were selected for their ability to produce acute mortality and the experiments designed to describe the influence of salinity change on that mortality. At present, we have no information on the salinity dependence of naphthalene exposure at realistic ambient concentrations. Our results suggest the potential for salinity effects in environmental naphthalene exposure of euryhaline fishes, and the physiological effects reported here serve as a data base for future analysis of the results of chronic sublethal exposure.

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American Public Health Association. 1971. Standard Methods for the Examination of Water and Wastewater, 13th ed. APHA, Washington, D.C. 874 p.

Anderson, J. W., J. M. Neff, B. A. Cox, H. E. Tatum, and G. M. Hightower. 1974. The effects of oil on estuarine animals: toxicity, uptake and depuration, respiration, p. 285–310. *In* F. J. Vernberg and W. B. Vernberg [ed.] Pollution and physiology of marine organisms. Academic Press, Inc., New York, N.Y.

Boylan, D. B., and B. W. Tripp. 1971. Determination of hydrocarbons in seawater extracts of crude oil and crude oil fractions. Nature 230: 44–47.

Burns, K. A., and D. Sabo. 1975. Environmental contamination and the induction of microsomal mixed function oxidases in an estuarine fish, *Fundulus heteroclitus*. (Abst.) Fed. Proc. 34: 810.

DiMichele, L., and M. H. Taylor. 1978. Histopathological and physiological responses of *Fundulus heteroclitus* to naphthalene exposure. J. Fish. Res. Board Can. 35: 1060–1066.

Eisler, R. 1965. Some effects of a synthetic detergent on estuarine fishes. Trans. Am. Fish. Soc. 94: 26–31.

Eisler, R., and P. H. Edmunds. 1966. Effects of endrin on blood and tissue chemistry of a marine fish. Trans. Am. Fish. Soc. 95: 153–159.

Eisler, R., and G. V. Kissil. 1975. Toxicities of crude oils and oil-dispersant mixtures to juvenile rabbitfish, *Siganus rivulatus*. Trans. Am. Fish. Soc. 104: 571–578.

Kinter, W. B., L. S. Merkens, R. H. Janicki, and A. M. Guarino. 1972. Studies of the mechanism of toxicity of DDT and polychlorinated biphenyls (PCBs): disruption of osmoregulation in marine fish. Environ. Health Perspec. 1: 169–173.

Kloth, T. C., and D. E. Wohlschlag. 1972. Size related metabolic responses of the pinfish, *Lagodon rhomboides*, to salinity variations and sublethal petrochemical pollution. Contrib. Mar. Sci. 16: 125–137.

Leach, G. J., and M. H. Taylor. 1977. Seasonal measurements of serum glucose and serum cortisol in a natural population of *Fundulus heteroclitus* L. Comp. Biochem. Physiol. 56A: 217–223.

Leadem, T. P., R. D. Campbell, and D. W. Johnson. 1974. Osmoregulatory response to DDT and varying salinities in *Salmo gairdneri*. I. Gill Na, K-ATPase. Comp. Biochem. Physiol. 49A: 197–204.

MORROW, J. E., R. L. GRITZ, AND M. P. KIRTON. 1975. Effects of some components of crude oil on young coho salmon. Copeia 1975: 326–331.

NEFF, J. M., AND J. W. ANDERSON. 1975. An ultraviolet spectrophotometric method for the determination of naphthalene and alkylnaphthalenes in the tissues of oil-contaminated marine animals. Bull. Environ. Contam. Toxicol. 14: 122–128.

NEFF, J. M., B. A. COX, D. DIXIT, AND J. W. ANDERSON. 1976. Accumulation and release of petroleum-derived aromatic hydrocarbons by four species of marine animals. Mar. Biol. 38: 279–289.

NORDLIE, F. G. 1978. The influence of environmental salinity on respiratory oxygen demands in the euryhaline teleost, *Ambassis interrupta* Bleeker. Comp. Biochem. Physiol. 59A: 271–274.

PICKFORD, G. E., F. B. GRANT, AND B. L. UMMINGER. 1969. Studies on the blood serum of the euryhaline cyprinodont fish, *Fundulus heteroclitus*. Trans. Conn. Acad. Arts Sci. 43: 25–70.

POTTS, W. T. W., AND D. H. EVANS. 1967. Sodium and chloride balance in the killifish *Fundulus heteroclitus*. Biol. Bull. 133: 411–425.

RICE, S. D., R. E. THOMAS, AND J. W. SHORT. 1977. Effect of petroleum hydrocarbons on breathing and coughing rates and hydrocarbon uptake-depuration in pink salmon fry, p. 259–277. *In* F. J. Vernberg, A. Calabrese, F. P. Thurberg, and W. B. Vernberg [ed.] Physiological responses of marine biota to pollutants. Academic Press, Inc., New York, N.Y.

SABO, D. J., J. J. STEGEMAN, AND L. S. GOTTLIEB. 1975. Petroleum hydrocarbon pollution and hepatic lipogenesis in the marine fish *Fundulus heteroclitus*. (Abst.) Fed. Proc. 34: 810.

SHELTON, G. 1970. The regulation of breathing, p. 293–359. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology, Vol. IV. Academic Press, Inc., New York, N.Y.

THOMAS, R. E., AND S. D. RICE. 1975. Increased opercular rates of pink salmon (*Oncorhynchus gorbuscha*) fry after exposure to the water-soluble fraction of Prudhoe Bay crude oil. J. Fish. Res. Board Can. 32: 2221–2224.

Acute Ammonia Toxicity and Ammonia Excretion in Rainbow Trout (*Salmo gairdneri*)

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HILLABY, B. A., AND D. J. RANDALL. 1979. Acute ammonia toxicity and ammonia excretion in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 36: 621–629.

Acute ammonia toxicity in rainbow trout (*Salmo gairdneri*) was studied by intraarterial injection of NH_4Cl and NH_4HCO_3. Hydrogen ion and total ammonia concentrations were measured in blood sampled from the dorsal aorta both before and after injection. Although injection of NH_4HCO_3 increased arterial blood pH, and injection of NH_4Cl decreased arterial blood pH, the same dose of each was required to kill fish. While the un-ionized form of ammonia in water has been shown to be toxic, in the blood either the ionized form or the total ammonia load is toxic to fish. Ammonia levels were measured in pre- and postbranchial blood. Mean values were not significantly different, but paired values indicated a fall in blood ammonia due to excretion across the gills. There appears to be a more rapid excretion of ammonia following NH_4HCO_3 infusions, which result in higher un-ionized ammonia levels in blood compared with those following NH_4Cl infusions. These results are consistent with the hypothesis that ammonia is excreted in the un-ionized form.

Key words: un-ionized ammonia, ionized ammonia, gills, pH, blood

HILLABY, B. A., AND D. J. RANDALL. 1979. Acute ammonia toxicity and ammonia excretion in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 36: 621–629.

Nous avons étudié la toxicité aiguë de l'ammoniac chez des truites arc-en-ciel (*Salmo gairdneri*) par injection intra-artérielle de NH_4Cl et NH_4HCO_3. Nous avons mesuré les concentrations d'ions hydrogène et d'ammoniac total dans le sang prélevé à même l'aorte dorsale avant et après injection. Bien que l'injection de NH_4HCO_3 élève le pH du sang artériel et que celle de NH_4Cl l'abaisse, il a fallu une même concentration des deux pour tuer les poissons. Bien qu'il ait été démontré que la forme non ionisée de l'ammoniac dans l'eau est toxique, une fois dans le sang, la forme ionisée ou la charge totale d'ammoniac est toxique pour les poissons. Nous avons mesuré les concentrations d'ammoniac dans le sang avant et après passage dans les branchies. Les valeurs moyennes ne diffèrent pas sensiblement mais les valeurs appariées indiquent une chute de l'ammoniac sanguin par suite de l'excrétion à travers les branchies. Cette excrétion semble se faire plus rapidement après infusion de NH_4HCO_3 qui entraîne une élévation des niveaux d'ammoniac non ionisé dans le sang, par comparaison à ce qui se produit après infusion de NH_4Cl. Ces résultats sont conformes à l'hypothèse que l'ammoniac est excrété sous la forme non ionisée.

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AMMONIA is found in natural waters from a variety of sources. It exists in solution as follows:

$$NH_3 + nH_2O \rightleftharpoons NH_3 \cdot nH_2O \rightleftharpoons NH_4^+ + OH^- + (n-1)H_2O$$

(Emerson et al. 1975). The concentration of un-ionized ammonia is dependent not only on total ammonia concentration, but also on pH, temperature, and ionic strength of a solution. The concentration of un-ionized ammonia increases with pH and temperature and decreases with an increase in ionic strength.

Ammonia is the major form of nitrogen excreted by teleost fish. It is produced in the liver (Pequin and Serfaty 1963; Vellas and Serfaty 1974) and excreted at the gills (Smith 1929; Wood 1958; Goldstein et al. 1964; Payan and Matty 1975). The kidney accounts for $<2\%$ of the total ammonia excreted by teleost fish (Smith 1929; Wood 1958; Fromm 1963; Fromm and Gillette 1968; Payan and Matty 1975). There is evidence that ammonia crosses the gills in both the ionized (Maetz and Garcia Romeu 1964; Maetz 1972, 1973; Payan and Maetz 1973; Payan and Matty 1975) and un-ionized forms (Goldstein et al. 1964; Fromm and Gillette 1968; Maetz 1973).

The toxic effects of ammonia in the water on fish have been related to the un-ionized form (Wuhrmann et al. 1947; Wuhrmann and Woker 1948; Smart 1975) due to its rapid entry into the fish. It is not clear, however, what the toxic action of ammonia is once it has entered the fish. Symptoms of ammonia toxicity in fish include hyperventilation, violent erratic movements including convulsions, and coma (Flis 1963a; Smart 1975). Prolonged exposure to sublethal levels of am-

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monia may cause a reduction in growth rate (Brockway 1950; Larmoyeux and Piper 1973), proliferation of gill lamellae, reduced stamina, and increased susceptibility to bacterial gill disease (Burrows 1964), thickening of the gill lamellae epithelium, reduction in lymphoid tissue in the spleen and hematopoietic tissue in the kidney (Larmoyeux and Piper 1973), tissue disintegration, lesions in blood vessels, and an abundant secretion of mucus (Flis 1963b).

Many external factors, such as temperature (Lloyd 1961), pH (Wuhrmann and Woker 1948; Smart 1975), dissolved carbon dioxide (Alabaster and Herbert 1954; Lloyd and Herbert 1960), dissolved oxygen (Downing and Merkens 1955; Merkens and Downing 1957; Larmoyeux and Piper 1973), and bacterial conversion of ammonia to nitrite (B.C. Research 1974) also contribute to toxic symptons observed in fish. These external factors may in themselves be toxic to fish or act synergistically with ammonia.

This study was designed to test the acute toxicity of ammonia to rainbow trout (*Salmo gairdneri*) by injecting directly into the dorsal aorta various concentrations of ammonia dissolved in isotonic saline. This procedure avoided complications due to many of the external factors that may act synergistically with ammonia on fish. The dorsal aorta, which is located postbranchially, was chosen as the site for injection because ammonia enters the fish at the gills.

Ammonia is also toxic to mammals (Jacquez et al. 1957; Warren and Schenker 1962; Wilson et al. 1968). Warren (1958) demonstrated a differential toxicity of ammonia to mice, depending on the ability of the ammonium salt to raise or lower blood pH. To test for a differential toxicity in fish, we designed these experiments to include in the injection solutions ammonium chloride, which lowered blood pH, and ammonium bicarbonate, which increased blood pH.

We also measured ammonia levels in ventral and dorsal aortic blood; these data were analyzed to determine whether ammonia was excreted across the gills in the ionized or un-ionized form.

Materials and Methods

Experiments were carried out on rainbow trout (*Salmo gairdneri*) of both sexes obtained from Sun Valley Trout Farm, Haney, British Columbia. Before experimentation fish were held outdoors in large circular tanks supplied with a flow of dechlorinated tapwater. They were fed daily with $\frac{5}{32}$-in. pellets of Clark's High Efficiency New Age Fish Feed. Water temperatures in the holding tank were between 6.0 and 13.8°C.

Dorsal aortic cannulations were performed after anesthetization by immersing in a 1:20 000 aqueous solution of the anesthetic MS-222®. After the aorta was punctured with a Medicut no. 16 (Sherwood Medical Industries Ltd.), the needle was removed, leaving a plastic sleeve in place. A cannula of Intramedic (Clay Adams, Inc.) polyethylene PE 50 tubing filled with heparinized (10 I.U. sodium heparin/mL) Cortland saline (Wolf 1963) was inserted through the sleeve, which was then removed. Glucose and phosphate were omitted from the Cortland saline in all cases. The cannula was secured with a stitch in the roof of the mouth, then the free end exited through a short collar of PE 190 tubing, which had been inserted in a hole punctured between the nares. Blood in the cannula was replaced with heparinized saline, and the free end of the cannula plugged with a metal pin. Ventral aortic cannulations were performed through the floor of the mouth by placing the cannula, of Intramedic polyethylene PE 50 tubing tipped with a no. 23 Yale needle and filled with heparinized Cortland saline, into the ventral aorta midway between the second and third gill arches. Blood in the cannula was replaced with heparinized saline, and the free end of the cannula was plugged with a pin and exited through a small hole cut between the branchiostegal rays. The cannula was secured with a stitch through the side of the mouth. If the ventral cannulation is performed before the dorsal one, there is a much less severe loss of blood. After cannulation, fish were placed in individual compartments in a covered black Perspex holding tank. A constant flow of air-saturated dechlorinated freshwater through the tank provided sufficient oxygen. Fish were allowed to recover from anesthesia and cannulation for at least a day before experimentation.

To test for toxicity, we injected 1 mL of an ammonia solution through the aortic cannula with a hand-held syringe, at a rate of 0.1 mL/min into the fish. Immediately before injection and 1 min following completion of the injection, 0.6-mL blood samples (0.5 mL for ammonia and 0.1 mL for pH analysis) were removed. Blood samples taken before injection were used to determine control levels of ammonia which were compared (paired *t*-test) with ammonia levels in blood samples taken from the same fish following ammonia infusion. The volume injected remained constant, but the ammonia concentration was varied.

After treatment to remove proteins (Williamson and Corky 1969), blood was analyzed at room temperature, with an Orion ammonia electrode, model 95-10, for total ammonia concentration. Calibration of the electrode for low level measurements of ammonia (Orion Research 1975) was performed daily at room temperature with a commercial standard solution (Orion) which has a stated accuracy to within ± 0.005 mol/L. Analysis at room temperature resulted in loss of $\sim 13\%$ of ammonia gas. The extent of ammonia loss was variable and therefore the data have not been corrected for this error. Thus our data are an underestimate of the actual ammonia level. Un-ionized and ionized ammonia concentrations were calculated from total ammonia concentration, pH, and temperature of the blood, using values taken from Trussell (1972) for percent un-ionized ammonia.

Measurements of blood pH were made with a Radiometer glass capillary electrode (G297/G2) connected to a Radiometer PHM 71 acid–base analyser. The electrode was calibrated and operated at the same temperature as the fish.

Student's *t*-test was used to compare differences between two means or for paired comparisons with a level of significance $P = 0.05$. All data, unless otherwise stated, are expressed as mean $\pm 95\%$ confidence limits. To determine the effects of ammonia-loading on ammonia excretion by trout, we carried out regression analysis on data previously analyzed for ammonia toxicity.

Experiments were also designed to determine whether ammonia toxicity varied with the length of period of re-

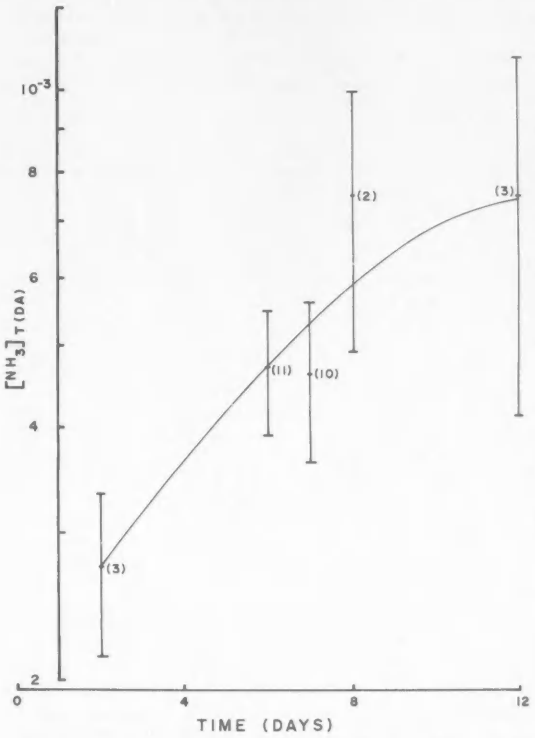

FIG. 1. Days following cannulation ($\simeq$ days of starvation) vs. total ammonia concentration (mol/dm^3 $\times$ 10^{-4}) of dorsal aortic blood (mean $\pm$ SE, n).

covery after cannulation. In some instances HCl instead of NH_4Cl was injected into the fish because NH_4Cl not only raises blood ammonia but also decreases blood pH, whereas HCl injections decrease blood pH but do not alter blood ammonia levels. Solutions of NH_4Cl or HCl dissolved in Cortland saline at various concentrations were injected into the dorsal aorta of rainbow trout (n = 34, body weight = 338.7 $\pm$ 27.7 g, temperature range = 6.0–12.5°C). The fish were observed for hyperventilation, loss of equilibrium, convulsions, and death. Death was indicated by complete cessation of ventilatory movements. Arterial blood pH (pH_a) and ammonia concentration were determined both before and 1 min after injection.

On the basis of the above experiments, fish were allowed a minimum of 5 d postoperative recovery before being injected with NH_4Cl, NH_4HCO_3, or HCl. Then solutions of different concentrations of NH_4Cl, NH_4HCO_3, or HCl in Cortland saline were injected into the dorsal aorta (n = 26; body weight = 315 $\pm$ 28.3 g; temperature range 9.0–12.5°C, but varying <0.5°C during any one experiment). The fish were observed for toxic effects; blood pH and ammonia levels were determined 1 min after completion of the injection. Blood pH, but not ammonia level, was measured at intervals following injection. Blood ammonia measurements were not carried out because of the large sample size required for their determination.

Results

Fish were not fed following cannulation and control dorsal aortic ammonia levels tended to increase during this period, probably as a result of starvation. There was considerable variability in the ammonia level, however, and hence values taken at different times were not significantly different (Fig. 1). Mean ventral and dorsal aortic blood ammonia levels were also not significantly different in resting, undisturbed, or cannulated trout. Total ammonia in ventral aortic blood was 4.09 $\pm$ 1.21 $\times$ 10^{-4} mol/dm^3 and in dorsal aortic blood, 5.09 $\pm$ 1.07 $\times$ 10^{-4} mol/dm^3 (Table 1). It appears, however, that variability between individual fish obscured any differences between dorsal and ventral aortic blood ammonia, because where paired values were determined simultaneously dorsal aortic blood always contained less total ammonia than ventral aortic blood (Table 2). Simultaneous cannulation of both dorsal and ventral aortae is difficult to achieve; only two large fish had patent cannulae in both vessels (Table 2 and 3). Table 3 contains values for ammonia extracted from the blood at the gills calculated from paired measurements, using a calculated cardiac output of 34.04 mL/kg-min (Wood 1974).

Fish allowed 5 d or more recovery time after cannulation survived an injection of either NH_4Cl or HCl whereas earlier injections affected or killed fish. There-

TABLE 1. Dorsal and ventral aortic blood ammonia concentrations and pH of undisturbed, cannulated rainbow trout. Blood samples were taken from fish with at least 1 d postoperative recovery. Numbers of fish given in parentheses. All values mean $\pm$95% confidence limits.

	$\sum[NH_3]^a$ mol/dm^3 ($\times$ 10^{-4})	$[NH_3]^b$ mol/dm^3 ($\times$ 10^{-6})	$[NH_4^+]^c$ mol/dm^3 ($\times$ 10^{-4})	pH	$[H^+]$ mol/dm^3 ($\times$ 10^{-8})	Body wt	Temp (°C)
Ventral aorta	4.09 $\pm$ 1.21 (7)	5.78 $\pm$ 3.66 (7)	4.03 $\pm$ 1.18 (7)	7.740 (7)	1.96 $\pm$ 0.748 (7)	395.9 $\pm$ 28.95	11.86 $\pm$ 1.12
Dorsal aorta	5.09 $\pm$ 1.07 (35)	4.90 $\pm$ 1.13 (35)	5.04 $\pm$ 1.06 (35)	7.674 (35)	2.21 $\pm$ 0.229 (35)	324.5 $\pm$ 23.47	11.03 $\pm$ 0.59

[a]Total ammonia.
[b]Un-ionized ammonia.
[c]Ionized ammonia.

TABLE 2. Paired dorsal and ventral aortic blood ammonia concentrations and pH of two intact, undisturbed, cannulated rainbow trout. Both fish had been allowed to recover for 1 d from the cannulation procedure. Ventral aortic and dorsal aortic samples were taken within a minute of each other. In one case the ventral sample was taken first, in the other the reverse was true.

Fish no. (body wt)		$\Sigma[NH_3]$ mol/dm³ ($\times 10^{-4}$)	$[NH_3]$ mol/dm³ ($\times 10^{-6}$)	$[NH_4^+]$ mol/dm³ ($\times 10^{-4}$)	pH	$[H^+]$ mol/dm³ ($\times 10^{-8}$)	Temp (°C)
1	Ventral aorta	5.83	9.56	5.73	7.84	1.41	13
(404 g)	Dorsal aorta	2.67	3.26	2.64	7.72	1.91	
2	Ventral aorta	5.83	10.9	5.72	7.97	1.07	11
(405 g)	Dorsal aorta	1.73	0.71	1.72	7.31	4.9	

TABLE 3. Ammonia extracted from blood at the gills of two rainbow trout based on data in Table 2 and assuming a cardiac output of 34.04 ml·kg^{-1}·min^{-1} (see text for details).

Fish no.	$\Sigma[NH_3]$ μmol/100 g-h	$[NH_3]$ μmol/100 g-h	$[NH_4^+]$ μmol/100 g-h
1	64.5	1.29	63.0
2	83.6	2.08	81.6

fore, we allowed fish a minimum of 5 d to recover after cannulation before any injections.

RESPONSES TO HCL INJECTIONS

Reaction to HCl, which started ~2 min from initiation of injection, consisted of jumping, erratic ventilation, and loss of equilibrium. Arterial blood pH (pH_a) was lowered below control values (Table 4) and blood appeared dark red to brown. Trout were unaffected when pH_a was reduced to 6.57 but lost equilibrium when it was reduced to 6.02. pH_a of surviving fish returned to normal levels within 40 min following the injection (Fig. 2). Data from one fish showed a total ammonia concentration in dorsal aortic blood of 3.79 $\times 10^{-4}$ mol/dm³ before injection of HCl, and 2.5 $\times$ 10^{-4} mol/dm³ following injection, both of which are within the range of control values (Table 5).

RESPONSES TO NH_4Cl INJECTIONS

Reaction to NH_4Cl, which started ~5 min from initiation of the injection, consisted of gulping at the

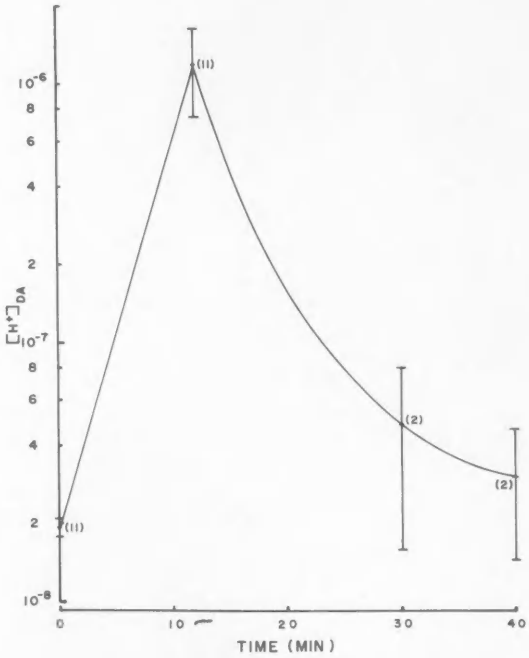

FIG. 2. Changes in hydrogen ion concentration (mol/dm³) of dorsal aortic blood with time following injection of HCl solution (mean ± SE, n). DA = dorsal aorta.

TABLE 4. Effect of HCl injections into the dorsal aorta of trout on fish survival and blood plasma pH, values mean ± 95% confidence limits.

		Blood pH			
		Before injection		After injection	
Effect of HCl injection on fish	Wt (g)	pH (avg value)	$[H^+]$ mol/dm³ $\times 10^{-8}$	pH (avg value)	$[H^+]$ mol/dm³ $\times 10^{-8}$
Not affected (n = 4)	372.1±120.3	7.711	2.01±0.848	6.537	59.1±11.3
Loss of equilibrium (n = 7)	303.0±82.1	7.740	1.88±0.563	6.020	151±166

TABLE 5. Dorsal aortic blood pH, H^+, and ammonia levels before (i) and after (f) injection of NH_4Cl and NH_4HCO_3. Values mean ± 95% confidence limits, except for pH where average values are given. $\sum[NH_3]$ = total ammonia concentration; $[NH_3]$ = un-ionized ammonia concentration.

	NH_4Cl-injected		NH_4HCO_3-injected		
	Alive (n = 7)	Dead (n = 4)	Alive (n = 3)	Affected (n = 7)	Dead (n = 5)
pH_i	7.711	7.610	7.646	7.676	7.739
pH_f	7.033	6.769	7.756	7.854	7.766
$[H^+]_i$	1.993±0.42	2.514±1.05	2.283±1.00	2.143±0.39	1.845±0.36
mol/dm³	× 10^{-8}	× 10^{-8}	× 10^{-8}	× 10^{-8}	× 10^{-8}
$[H^+]_f$	9.997±3.09	1.743±0.64	1.760±0.44	1.404±0.12	1.759±0.53
mol/dm³	× 10^{-8}	× 10^{-7}	× 10^{-8}	× 10^{-8}	× 10^{-8}
$\sum[NH_3]_i$	5.934±3.75	6.003±4.67	7.53±11.8	4.29±1.93	5.10±4.05
mol/dm³	× 10^{-4}	× 10^{-4}	× 10^{-4}	× 10^{-4}	× 10^{-4}
$\sum[NH_3]_f$	2.146±0.40	3.92±1.07	1.78±2.20	2.75±1.21	2.74±0.89
mol/dm³	× 10^{-2}	× 10^{-2}	× 10^{-2}	× 10^{-2}	× 10^{-2}
$[NH_3]_i$	5.62±4.30	5.55±5.44	6.57±9.67	4.44±2.13	5.72±5.15
mol/dm³	× 10^{-6}	× 10^{-6}	× 10^{-6}	× 10^{-6}	× 10^{-6}
$[NH_3]_f$	4.38±1.68	4.72±2.68	3.82±9.44	4.26±2.02	3.61±2.52
mol/dm³	× 10^{-5}	× 10^{-5}	× 10^{-4}	× 10^{-4}	× 10^{-4}
Wt (g)	355.4±73.0	311.9±47.7	335.5±211.5	293.6±69.8	283.5±70.9
Dose (μmol/100 g)	246.1±65.1	522.5±117.7	253.7±106.6	342.4±55.3	409.4±166.6

surface, coughing, increased ventilation followed by sporadic ventilation, loss of equilibrium, and muscle spasms which ended in violent convulsions and death. pH_a was lowered below, and total ammonia concentration was increased above, control values (Table 5). Blood appeared dark red. pH_a of surviving fish returned to normal levels within 1 h following the injection (Fig. 3).

Final blood total ammonia concentration, which was not significantly different from ammonium ion concentration, was greater in fish that died than in those that survived (Fig. 4). There was no significant difference in blood un-ionized ammonia concentration between fish that lived or those that died (Fig. 5). Total ammonia concentration was not followed after injection because removal of such large blood volumes may have resulted in death of the fish.

The hematocrit of 22.8 ± 7.62% before injection of NH_4Cl was not significantly different from that of 23.2 ± 2.46% following injection. A blood sample taken following injection and examined under the light microscope showed no lysing of red blood cells.

RESPONSES TO NH_4HCO_3 INJECTIONS

Reaction to NH_4HCO_3, which started ~8 min from initiation of the injection, consisted of loss of equilibrium and muscle spasms which sometimes led to violent convulsions and death. The color of some fish changed noticeably, from normal, to quite dark, to very light, and back to normal again if the fish recovered. Some fish also had noticeably protruding eyes. pH_a and blood total ammonia concentration were increased above control values (Table 5). Blood appeared bright red.

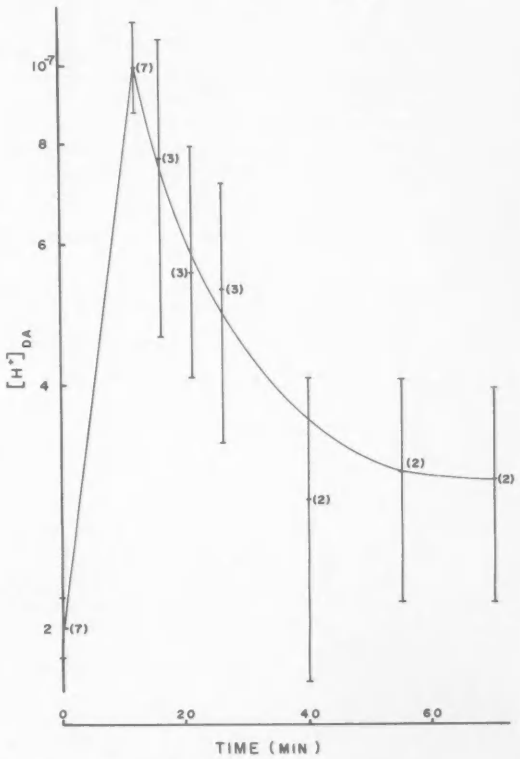

FIG. 3. Changes in hydrogen ion concentration (mol/dm³) of dorsal aortic blood with time following injection of NH_4Cl solution (mean ± SE, n). DA = dorsal aorta.

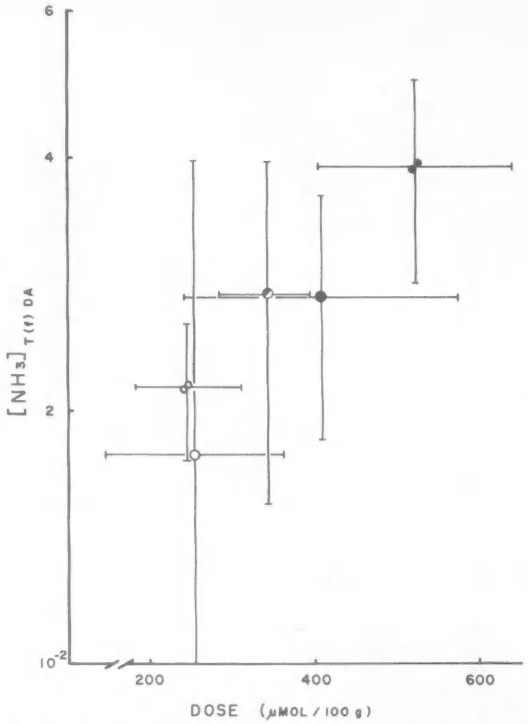

FIG. 4. Dose of ammonium salt injected vs. total ammonia concentration (mol/dm^3) of dorsal aortic blood following injection. Values expressed as mean ± 95% confidence limits. NH_4HCO_3 injection: ○ fish survived, ◔ fish affected, ● fish died; NH_4Cl injection: ♂ fish survived, ♂ fish died.

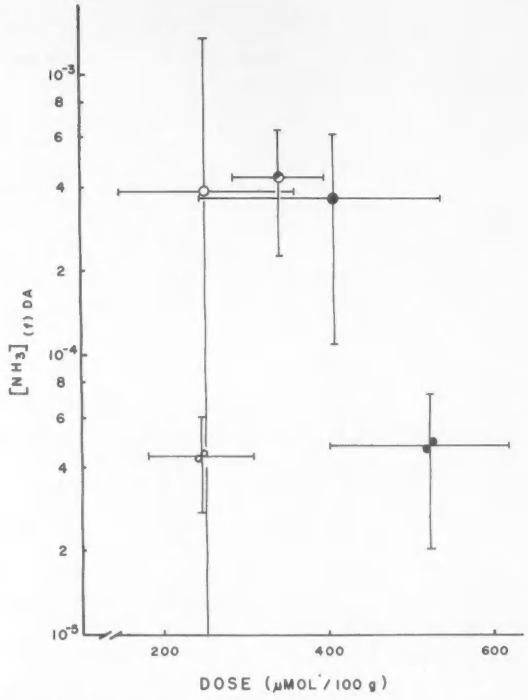

FIG. 5. Dose of ammonium salt injected vs. concentration of un-ionized ammonia (mol/dm^3) of dorsal aortic blood following injection. (Symbols as described in Fig. 4). Values expressed as mean ±95% confidence limits.

Injection of 522.4 ± 117.7 μmol NH_4Cl/ 100 g killed fish and was not significantly different than the injection of 409.4 ± 166.6 μmol NH_4HCO_3/100 g which killed fish (Table 5). Final blood total and ionized ammonia concentration in fish that died from NH_4Cl injection were higher than from NH_4HCO_3 injection (Fig. 4), but the opposite was true for final blood un-ionized ammonia concentration (Fig. 5, Table 5).

Regression analysis of data obtained from injecting NH_4Cl and NH_4HCO_3 solutions into trout demonstrated two things. First, blood ammonia concentration is related to dose following only NH_4Cl injection (Fig. 6), and not following NH_4HCO_3 injection. The regression line for NH_4HCO_3 was not plotted because the slope was not significantly different than 0. Second, while ammonia excreted is a function of dose injected, the slope of the regression line related to NH_4HCO_3 injection is significantly ($P = 0.05$) steeper than that related to NH_4Cl injection (Fig. 7).

Discussion

Injections of Cortland saline with or without elevated chloride levels have no measurable effect on trout as long as the rate of injection is slow and the volume of injection is small (<1% of body volume) (Janssen and Randall 1975). An increase in saline bicarbonate levels causes a marked rise in blood pH but the fish is able to restore normal blood pH levels rapidly with no apparent detrimental effects on the fish (Janssen and Randall 1975). In this study we have shown that injections of HCl, which decrease pH but do not affect blood ammonia levels, can kill fish. The pH drop required to kill the fish, however, is much larger than that seen following ammonium chloride injections. Thus the mortality following ammonia injection cannot be the result of only the associated acidosis. In fact, similar doses of NH_4Cl and NH_4HCO_3 were required to kill fish. These two ammonium salts have very different effects on blood pH, indicating that toxicity is due mainly to elevated ammonia levels in the blood rather than the changes in $[H^+]$, $[Cl^-]$, and $[HCO^-_3]$ associated with these injections. The variability in our results, however, prevents any categoric statement, and there may be some detrimental synergistic action of $[H^+]$ and $[NH^+_4]$ on trout.

Symptoms observed in rainbow trout following injec-

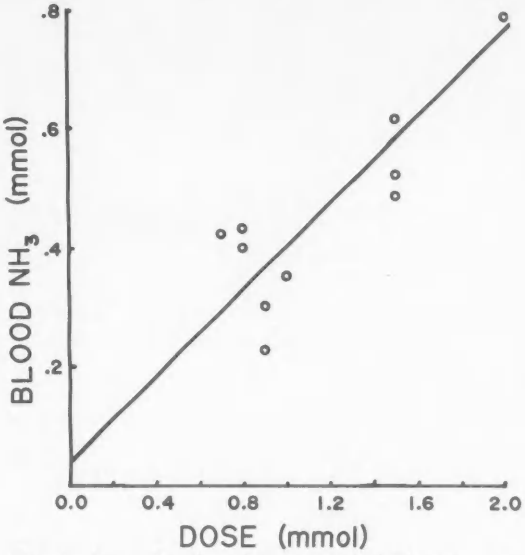

FIG. 6. Regression line of dose of NH_4Cl injected vs. ammonia remaining in blood from injection ($Y = 0.04241 + 0.3618X$).

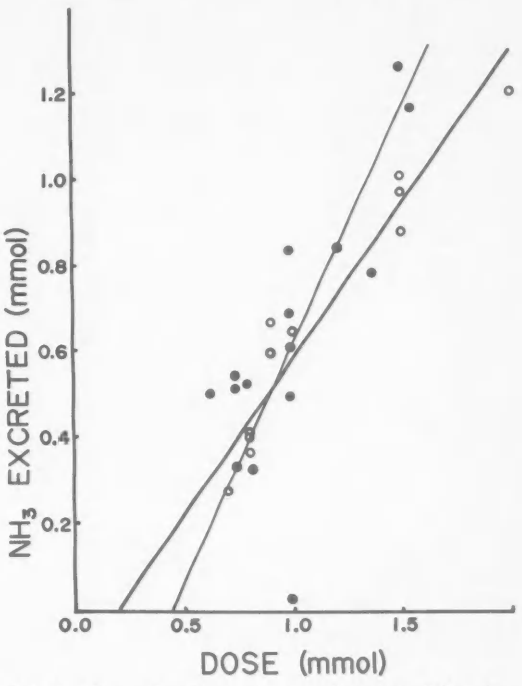

FIG. 7. Regression lines of ammonium dose injected vs. ammonia excreted. NH_4Cl: $Y = -0.1401 + 0.7248X$ (○); NH_4HCO_3: $Y = -0.4856 + 1.106X$ (●) (steeper slope).

tion of either NH_4Cl or NH_4HCO_3 are consistent with those observed in other animals (Warren 1958; Flis 1963a; Wilson et al. 1968, 1969; Smart 1975) as a result of ammonia toxicity. There was no significant difference in the injection dose of NH_4Cl or NH_4HCO_3 that killed fish; however, there was significantly more un-ionized ammonia in the NH_4HCO_3 solution. Therefore, in fish, unlike mammals (Warren 1958; Warren and Schenker 1962), there is no increased toxicity as a result of increased un-ionized ammonia caused by an increase in blood pH. Thus, in trout it appears that either the ionized fraction or the total ammonia load, but not ammonia gas levels, within the fish is exerting the toxic effects.

Ammonia appears to affect neuronal activity, as indicated by the convulsion observed in this experiment and others. This could be the result of the disruption of ion transport by ammonia or more generalized effects like the lowering of ATP levels in the brain as observed by Smart (1975). To affect brain function, ammonia must cross the blood–brain barrier. The nature and extent of this barrier may account for the differential effect of pH on toxicity in fish and mammals, the barrier being much more extensive in mammals, with movement of ammonia depending largely on the diffusion of the un-ionized molecule.

The response to NH_4Cl was more rapid than that to NH_4HCO_3. Toxic effects were seen 5.3 ± 0.88 min after the onset of infusion of NH_4Cl whereas 9.1 ± 2.0 min elapsed before there was any response to NH_4HCO_3. It took 10 min to infuse the ammonia dose into each fish, and toxic effects were seen in the majority of fish while NH_4^+ was being infused. Ammonia is removed from the blood more rapidly following NH_4HCO_3 infusion (see below) so the time taken to reach the toxic dose in whole blood would be longer following NH_4HCO_3 infusion. Thus the time delay between the onset of infusion and the appearance of signs of toxicity could be related to the time taken for toxic levels to accumulate in the blood.

Smith (1929) clearly demonstrated that the gills are the major site of ammonia excretion in teleost fish. Subsequent experiments (Wood 1958; Fromm 1963; Fromm and Gillette 1968; Payan and Matty 1975) support these findings and indicate that the kidney accounts for $<2\%$ of the total ammonia excreted.

Our measurements of blood ammonia (Table 2) show a significant decrease in blood ammonia between ventral and dorsal aortae and add support to the hypothesis that most, if not all, of the ammonia excreted at the gills comes from the blood. Calculations of ammonia extracted from the blood mean of 74 μ mol·100 g of fish^{-1}·h^{-1} (Table 3) are within the range 100 to 49 μmol·100 g of fish^{-1}·h^{-1} calculated from data from Fromm (1963); i.e. 106.8 to 54.9 μmol·100 g^{-1}·h^{-1} corrected for basal excretion (5.9 μmol·100 g^{-1}·h^{-1}) (Payan and Matty 1975) for the same species under similar conditions.

Although ammonia is excreted at the gills, it is not clear whether it is excreted in the un-ionized or ionized form. It has been postulated that NH_4^+ is the main form excreted, with NH_3 excretion occurring in the absence of external Na^+, or at low external pH (Maetz and Garcia Romeu 1964; Maetz 1972, 1973; Payan and Maetz 1973; Payan 1978).

Our results provide evidence for ammonia excretion in the un-ionized form. Following NH_4HCO_3 injection, arterial blood pH increases, resulting in a larger proportion of blood ammonia being in the un-ionized form. The opposite occurs following NH_4Cl injection. The slopes of the regression lines (Fig. 7) indicate that ammonia is excreted more rapidly following NH_4HCO_3 injection. Excretion of un-ionized ammonia could account for this difference. Also, although blood ammonia increases with dose following NH_4Cl injection, no such relationship exists between blood ammonia and dose of NH_4HCO_3 (Fig. 6), as ammonia appears to be removed from the blood more rapidly following NH_4HCO_3 injection.

Further evidence for rapid movement of NH_3 across the gills is given by Wuhrmann and Woker (1948) who found that NH_3 rather than NH_4^+ in water was toxic to fish. They argued that NH_3 and not NH_4^+ crossed the gill epithelium readily. Further support of this hypothesis is given by Smart (1975). Following exposure of trout to the same levels of ammonia in water, Smart measured a more rapid increase in blood ammonia at elevated water pH levels. Thus we conclude that although NH_4^+ may be coupled to sodium influx, in general, un-ionized ammonia is the major form of ammonia excreted across the gills.

The problem of toxicity from ammonia in water is compounded by many factors; however, it appears that in water the un-ionized form exerts toxic effects by entry into the fish at the gills. This study indicates that the un-ionized form in fish is not acutely toxic, but that either the ionized form or the total ammonia load is toxic.

Acknowledgments

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ALABASTER, J. S., AND D. W. M. HERBERT. 1954. Influence of carbon dioxide on the toxicity of ammonia. Nature 174: 404.

B.C. RESEARCH. 1974. Feasibility and design studies for water reclamation and waste water treatment at a trout hatchery. Prepared for British Columbia Department of Public Works by Division of Applied Biology, B.C. Research. Vancouver, B.C. 234 p.

BROCKWAY, D. R. 1950. Metabolic products and their effects. Prog. Fish-Cult. 12: 127–129.

BURROWS, R. E. 1964. Effects of accumulated excretory products on hatchery-reared salmonids. U.S. Fish. Wildl. Serv. Bur. Sport Fish. Wildl. Res. Rep. 66: 1–12.

DOWNING, K. M., AND J. C. MERKENS. 1955. The influence of dissolved oxygen concentration on the toxicity of unionized ammonia to rainbow trout (*Salmo gairdneri* Richarson). Ann. Appl. Biol. 43: 243–246.

EMERSON, K., R. C. RUSSO, R. E. LUND, AND R. V. THURSTON. 1975. Aqueous ammonia equilibrium calculations: Effect of pH and temperature. J. Fish. Res. Board Can. 32: 2379–2383.

FLIS, J. 1963a. Anatomicohistopathological changes induced in carp (*Cyprinus carpio* L.) by ammonia water. I. Effects of toxic concentrations. Acta Hydrobiol. 10: 205–224.

1963b. Anatomicohistopathological changes induced in carp (*Cyprinus carpio* L.) by ammonia water. II. Effects of subtoxic concentrations. Acta Hydrobiol. 10: 225–238.

FROMM, P. O. 1963. Studies on renal and extra-renal excretion in the fresh-water teleost, *Salmo gairdneri*. Comp. Biochem. Physiol. 10: 121–128.

FROMM, P. O., AND J. R. GILLETTE. 1968. Effect of ambient ammonia on blood ammonia and nitrogen excretion of rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 26: 887–896.

GOLDSTEIN, L., R. FORSTER, AND G. FANELLI JR. 1964. Gill blood flow and ammonia excretion in the marine teleost, *Myoxocephalus scorpius*. Comp. Biochem. Physiol. 12: 489–499.

JANSSEN, R. G., AND D. J. RANDALL. 1975. The effects of changes in pH and P_{CO_2} in blood and water on breathing in rainbow trout, *Salmo gairdneri*. Respir. Physiol. 25: 235–245.

JACQUEZ, J. A., J. W. POPPELL, W. LAWRENCE JR., AND K. E. ROBERTS. 1957. Clinical significance of the partial pressure of ammonia (P_{NH_3}) in patients with ammonia toxicity (abstract). Clin. Res. Proc. 5: 20.

LARMOYEUX, J. D., AND R. G. PIPER. 1973. Effects of water reuse on rainbow trout in hatcheries. Prog. Fish Cult. 35: 2–8.

LLOYD, R. 1961. The toxicity of ammonia to rainbow trout (*Salmo gairdneri* Richardson). Water Waste Treat. J. 8: 278–279.

LLOYD, R., AND D. W. M. HERBERT. 1960. The influence of carbon dioxide on the toxicity of unionized ammonia to rainbow trout (*Salmo gairdneri* Richardson). Ann. Appl. Biol. 48: 399–404.

MAETZ, J. 1972. Branchial sodium exchange and ammonia excretion in the goldfish *Carassius auratus*. Effects of ammonia-loading and temperature. J. Exp. Biol. 56: 601–620.

1973. Na^+/NH_4^+ exchanges and NH_3 movement across the gill of *Carassius auratus*. J. Exp. Biol. 58: 255–275.

MAETZ, J., AND F. GARCIA ROMEU. 1964. The mechanism of sodium and chloride uptake by the gills of freshwater fish, *Carassius auratus*. II. Evidence for NH_4^+/Na^+ and HCO_3^-/Cl^- exchanges. J. Gen. Physiol. 47: 1209–1226.

MERKENS, J. C., AND K. M. DOWNING. 1957. The effect of tension of dissolved oxygen on the toxicity of unionized ammonia to several species of fish. Ann. Appl. Biol. 45: 521–527.

ORION RESEARCH. 1975. Instruction Manual — ammonia electrode model 95-10. Orion Research Incorporated. Cambridge, Mass. 28 p.

PAYAN, P. 1978. A study of the Na^+/NH_4^+ exchange across the gill of the perfused head of the trout (*Salmo gairdneri*). J. Comp. Physiol. 124: 181–188.

PAYAN, P., AND J. MAETZ. 1973. Branchial sodium transport mechanisms in *Scyliorhinus canicula*: evidence for

Na^+/NH_4^+ and Na^+/H^+ exchanges and for a role of carbonic anhydrase. J. Exp. Biol. 58: 487–502.

PAYAN, P., AND A. J. MATTY. 1975. The characteristics of ammonia excretion by a perfused isolated head of trout (*Salmo gairdneri*): effect of temperature and CO_2-free Ringer. J. Comp. Physiol. 96: 167–184.

PEQUIN, L., AND A. SERFATY. 1963. L'excretion ammoniacale chez un téléostéen dulciocle: *Cyprinus carpio L.* Comp. Biochem. Physiol. 10: 315–324.

SMART, G. R. 1975. The acute toxic mechanisms of ammonia to rainbow trout (*Salmo gairdneri*). Ph.D. Thesis. University of Bristol. U.K.

SMITH, H. W. 1929. The excretion of ammonia and urea by the gills of fish. J. Biol. Chem. 81: 727–742.

TRUSSELL, R. P. 1972. The percent unionized ammonia in aqueous ammonia solutions at different pH levels and temperatures. J. Fish. Res. Board Can. 29: 1505–1510.

VELLAS, F., AND A. SERFATY. 1974. L'ammoniaque et l'urée chez un Téleostéen d'eau douce: la Carpe (*Cyprinus carpio L.*). J. Physiol. Paris 68: 591–614.

WARREN, K. S. 1958. The differential toxicity of ammonium salts. J. Clin. Invest. 37: 497–501.

WARREN, K. S., AND S. SCHENKER. 1962. Differential effect of fixed acid and carbon dioxide on ammonia toxicity. Am. J. Physiol. 203: 903–906.

WILLIAMSON, J. R., and B. C. CORKY. 1969. Assays of intermediates of the citric acid cycle and related compounds by fluorometric enzyme methods. Meth. Enzym. 13: 434–511.

WILSON, R. P., R. O. ANDERSON, AND R. A. BLOOMFIELD. 1969. Ammonia toxicity in selected fishes. Comp. Biochem. Physiol. 28: 107–118.

WILSON, R. P., M. E. MUHRER, AND R. A. BLOOMFIELD. 1968. Comparative ammonia toxicity. Comp. Biochem. Physiol. 25: 295–301.

WOLF, K. 1963. Physiological salines for freshwater teleosts. Prog. Fish-Cult. 25: 135–140.

WOOD, C. M. 1974. Mayer waves in the circulation of a teleost fish. J. Exp. Zool. 189: 267–274.

WOOD, J. D. 1958. Nitrogen excretion in some marine teleosts. Can. J. Biochem. Physiol. 36: 1237–1242.

WUHRMANN, K., AND H. WOKER. 1948. Beiträge zur Toxikologie der Fische. II. Experimentelle Untersuchungen über die Ammoniak- und Blausäurevergiftung. Schweiz. Z. Hydrol. 11: 210–244.

WUHRMANN, K., F. ZEHENDER, AND H. WOKER. 1947. Über die fischereibiologische Bedeutung des Ammonium — und Ammoniakgehaltes fliessender Gewässer. Vierteljahrsschr. Naturforsch, Ges. Zürich 92: 198–204.

Karyotypic and Evolutionary Relationships of the Yellowstone (*Salmo clarki bouvieri*) and West-Slope (*S. c. lewisi*) Cutthroat Trout

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LOUDENSLAGER, E. J., AND G. H. THORGAARD. 1979. Karyotypic and evolutionary relationships of the Yellowstone (*Salmo clarki bouvieri*) and west-slope (*S. c. lewisi*) cutthroat trout. J. Fish. Res. Board Can. 36: 630–635.

Yellowstone cutthroat trout (*Salmo clarki bouvieri*) have 64 chromosomes, without any subtelocentric chromosomes, while west-slope cutthroat trout (*S. c. lewisi*) have 66 chromosomes, including 12 subtelocentrics. Both subspecies have 104 chromosome arms. Chromosomal similarities are apparent between the Yellowstone cutthroat and Great Basin cutthroat subspecies, and between west-slope cutthroat trout and coastal cutthroat trout. These results demonstrate that at least two distinct evolutionary lines of cutthroat trout are present in the northern Rocky Mountain region.

Key words: Salmo clarki, karyotypes, Yellowstone cutthroat trout, west-slope cutthroat trout, evolutionary relationships

LOUDENSLAGER, E. J., AND G. H. THORGAARD. 1979. Karyotypic and evolutionary relationships of the Yellowstone (*Salmo clarki bouvieri*) and west-slope (*S. c. lewisi*) cutthroat trout. J. Fish. Res. Board Can. 36: 630–635.

La truite fardée (*Salmo clarki bouvieri*) de Yellowstone possède 64 chromosomes, sans aucun subtélocentrique, alors que celle du versant occidental (*S. c. lewisi*) a 66 chromosomes, y compris 12 subtélocentriques. L'une et l'autre sous-espèce ont 104 bras de chromosomes. Il existe des ressemblances évidentes entre les sous-espèces de truites fardées de Yellowstone et celles de Great Basin, d'une part, et entre les truites fardées du versant occidental et celles des eaux côtières, d'autre part. Ces résultats démontrent qu'il existe au moins deux lignées évolutionnaires de truites fardées dans la région septentrionale des montagnes Rocheuses.

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THE systematics of inland populations of cutthroat trout (*Salmo clarki*) remains unclear despite previous investigations (Miller 1950; Needham and Gard 1959; Behnke 1965). Several factors have contributed to the confusion: the introduction of rainbow trout (*S. gairdneri*) and nonnative cutthroat trout (often the Yellowstone cutthroat) has reduced the number of pure native populations and complicated their identification; recent geological events have complicated our understanding of the dispersal and present zoogeography of the existing subspecies; and the range of meristic and morphological variability may be as great within a region as between regions.

Inland cutthroat trout of the upper Columbia River drainage and the headwaters of the South Saskatchewan, Missouri, and Yellowstone rivers form one of the confusing groups. The designation *S. clarki lewisi* has sometimes been applied to all of these cutthroat trout in the past (Schultz 1935; Behnke 1965). More recently the cutthroat trout of Yellowstone Lake and River and the large-spotted cutthroat trout of the Snake River above Shoshone Falls have been considered a separate subspecies, *S. c. bouvieri* (Yellowstone cutthroat trout) (Behnke 1973, 1975, and personal communication, Colorado State University, Fort Collins). The designation *S. c. lewisi* (west-slope cutthroat trout) may now apply only to native cutthroat trout in the South Saskatchewan, Missouri, and upper Columbia River systems, with the exception of the Snake River above Shoshone Falls (Behnke 1973, 1975). Because of their availability and early association with the name *S. c. lewisi*, Yellowstone cutthroat trout were widely propagated and introduced throughout much of the range of west-slope cutthroat trout from 1900 to 1950 (Behnke 1973). There is, however, little information available on genetic differences

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TABLE 1. Chromosome numbers in somatic cells of Yellowstone and west-slope cutthroat trout.

Population	Common name	Number of fish	Cells with $2n=$										
			58	59	60	61	62	63	64	65	66	67	68
Le Hardy's	Yellowstone	3	—	—	2	—	5	1	23	2	11	—	—
Pelican Creek	Yellowstone	7	—	1	1	1	1	3	46	2	6	—	—
Snake River	Yellowstone	21	3	—	11	—	18	1	83	1	8	—	—
Dime Creek	Yellowstone	10	9	—	11	—	7	5	42	—	1	—	—
Jocko Hatchery	West-slope	5	—	4	—	—	1	2	—	3	47	—	2

between these subspecies or the potential consequences of past mixing and hydridization.

Miller (1972) and Gold (1977) suggest that detailed comparative studies of karyotypes may clarify the origins and relationships among western *Salmo*. Karyotype differences between coastal and inland cutthroat trout have been described previously (Simon 1964; Gold et al. 1977). In this report, we describe the karyotypes of the Yellowstone and west-slope cutthroat trout and discuss their systematic implications.

Materials and Methods

Yellowstone cutthroat trout were sampled from Le Hardy's Rapids (Yellowstone River) and Pelican Creek within the Yellowstone drainage, Yellowstone National Park, Wyoming. Within the Snake River drainage, Yellowstone cutthroat trout were sampled from Dime Creek and the Snake River near the Jackson Hole Biological Research Station, Grand Teton National Park, Wyoming. West-slope cutthroat trout were obtained from the Jocko River State Trout Hatchery, Arlee, Montana. This stock originated from the Hungry Horse drainage (Flathead River system), Montana, and is believed to represent pure west-slope cutthroat trout.

Chromosome preparations of Yellowstone cutthroat trout followed the method of Gold (1974) with modification. Briefly, homogenized anterior head kidney was treated with a 0.075 N KCl hypotonic solution and fixed in Carnoy's solution (3:1 methanol:glacial acetic acid). One or two drops of the fixed cell suspension were dropped onto cold wet (distilled water) microscope slides which were then plate dried. Chromosome preparations of west-slope cutthroat trout were prepared from white blood cell cultures as previously described (Thorgaard 1976). Slides were stained in dilute (4–6%) Giemsa in phosphate buffer or C-banded using the $Ba(OH)_2$ technique (Salamanca and Armendares 1974).

Chromosome variation within individual salmonids has been reported (Ohno et al. 1965; and others). However, our comparison of kidney chromosome preparations with white blood cell culture preparations is justified because the same modal chromosome numbers have been obtained for these two tissues in redband trout (Wilmot 1974; Gold 1977), hatchery strains of rainbow trout (Heckman et al. 1971; Fukuoka 1972a), and sockeye salmon (Fukuoka 1972b; Thorgaard 1978).

Results and Discussion

Chromosome number and morphology of 41 Yellowstone cutthroat trout from 4 locations and 5 west-slope cutthroat trout from the Jocko River Trout Hatchery were determined (Table 1).

Of the 305 cells of Yellowstone cutthroat trout analyzed, 64% (194) were modal with $2n = 64$, 26% (80) were hypomodal, and 10% (31) were hypermodal. This variation is within the range of error typically observed for salmonid chromosome preparations (Thorgaard 1976; Gold 1977; Gold et al. 1977) and is attributed to chromosome loss during preparation and counting errors caused by early chromatid separation. All individuals were modal with $2n = 64$, and approximately equal numbers of cells were counted per individual. The modal karyotype (Fig. 1) is characterized by 38 metacentric chromosomes, two of which often have satellites (M; pairs 1–19), 2 small submetacentric chromosomes (SM; pair 20), 0 subtelocentric chromosomes (ST), and 24 acrocentric chromosomes (A; pairs 21–32). The chromosome arm number (NF) is 104. This karyotype is indistinguishable from the karyotype of an undescribed subspecies of cutthroat trout from the Alvord Basin, Oregon, and similar to the Lahontan cutthroat trout, *S. c. henshawi* (Table 2).

Of the 59 cells analyzed from west-slope cutthroat trout 80% (47) were modal with $2n = 66$, 17% (10) were hypomodal, and 3% (2) were hypermodal. All individuals were modal with $2n = 66$. The modal karyotype (Fig. 2) is characterized by 34 metacentric chromosomes (pairs 1–17), 4 small submetacentric chromosomes (pairs 18 and 19), 12 subtelocentric chromosomes (pairs 20–25), and 16 acrocentric chromosomes (pairs 26–33). Using the criteria that only metacentric and submetacentric chromosomes are scored as biarmed, the NF is estimated to be 104. This karyotype has not been described previously for any *Salmo*, but closely resembles the coastal cutthroat trout (*S. c. clarki*) karyotype because of the many subtelocentric chromosomes and higher chromosome number (Table 2).

There is an apparent discrepancy between the karyotype we report and that reported by Simon and Dollar (1963) and Simon (1964) for *S. c. lewisi* (Table 2). The chromosome number difference can be accounted for if Simon karyotyped Yellowstone cutthroat trout, which at that time were considered part of the

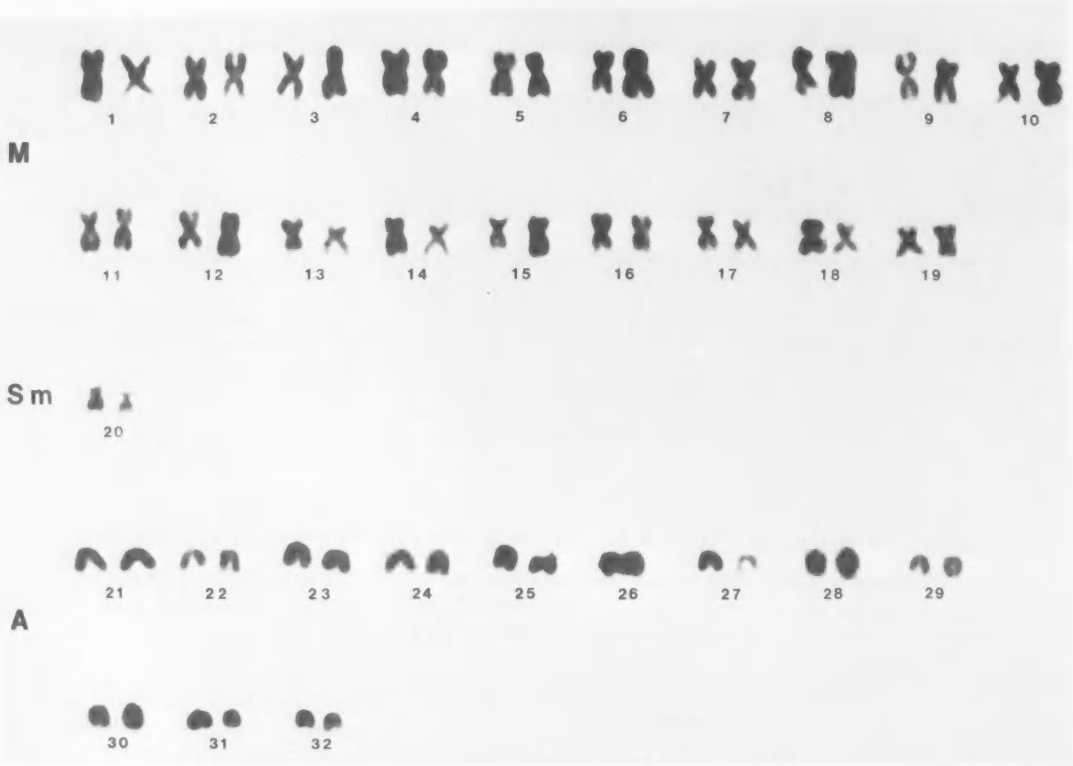

FIG. 1. Karyotype of $2n = 64$ Yellowstone cutthroat trout from Le Hardy's Rapids–Yellowstone River. M indicates metacentric chromosomes, Sm indicates submetacentric chromosomes, and A indicates acrocentric chromosomes.

S. c. lewisi subspecies. However, in addition to observing inland cutthroat trout with 64 chromosomes, Simon (1964) observed cutthroat trout with 64, 65, and 66 chromosomes from Lauri Lake, Montana. Behnke (personal communication) has informed us that Lauri Lake was stocked with Yellowstone × west-slope hybrids in 1958. Simon's observations thus provide further evidence of individuals with 66 chromosomes in populations of cutthroat trout in the upper Columbia system. The arm number inconsistency between our results and Simon's could result from difficulties in centromere placement in Simon's embryo squashes (Gold et al. 1977).

C-banded preparations of both Yellowstone and west-slope cutthroat trout revealed only centric constitutive heterochromatin. Thus the minor elements on the ST

TABLE 2. Comparison of karyotypes of *Salmo clarki* subspecies.

Subspecies	Common name	2n	NF	M	SM	ST	A	Source
S. c. subspecies	Alvord cutthroat	64	104	38	2	0	24	Wilmot (1974)
S. c. henshawi	Lahontan cutthroat	64	104	36	4	0	24	Gold et al. (1977)
S. c. bouvieri	Yellowstone cutthroat	64	104	38	2	0	24	This study
S. c. clarki[a]	Coast cutthroat	68	104	36	0	18	14	Gold et al. (1977)
S. c. clarki	Coast cutthroat	70	106	38	0	32	0	Simon (1964)
S. c. lewisi[b]	West-slope cutthroat	64	106	42	?	?	22	Simon and Dollar (1963); Simon (1964)
S. c. lewisi	West-slope cutthroat	66	104	34	4	12	16	This study

[a]Numbers of ST and A chromosomes estimated from figures presented in Gold et al. (1977).

[b]Simon and Dollar (1963) and Simon (1964) combined metacentric and submetacentric chromosomes together as metacentric, and subtelocentric and acrocentric together as acrocentric.

FIG. 2. Karyotype of $2n = 66$ west-slope cutthroat trout from the Jocko River Trout Hatchery. M indicates metacentric chromosomes, Sm indicates submetacentric chromosomes, St indicates subtelocentric chromosomes, and A indicates acrocentric chromosomes.

chromosomes do not appear to be constitutive heterochromatin, which sometimes accounts for polymorphisms of this type in small mammals such as *Peromyscus* (Murray and Kitchin 1976) and *Thomomys* (Patton and Yang 1977).

The karyotypes of west-slope cutthroat trout from the Jocko River Trout Hatchery and Yellowstone cutthroat trout differ by a diploid chromosome number of two, without a change in arm number. The simplest explanation for this difference is the fusion of two pairs of acrocentric or subtelecentric chromosomes, or the dissociation of a pair of bi-armed chromosomes. There is also a difference in the number of small submetacentric chromosomes. In addition, west-slope cutthroat trout are characterized by 12 subtelocentric chromosomes, including one distinctive long pair (pair 20, Fig. 2), but there are no subtelocentric chromosomes in the Yellowstone cutthroat trout.

Because attempts to G-band trout chromosomes have thus far failed, there is no way to assess additional chromosomal homologies between west-slope and Yellowstone cutthroat trout. However, these gross karyotypic differences suggest that substantial chromosomal differentiation is present between west-slope and Yellowstone cutthroat trout.

A comparison of Yellowstone and west-slope cutthroat karyotypes to those available for other *S. clarki* subspecies (Table 2) suggests some very interesting affinities. The Yellowstone karyotype is similar to that of two Great Basin subspecies, the Alvord cutthroat trout and the Lahontan cutthroat trout. The west-slope karyotype, although unique, is most easily derived from that of the coastal cutthroat trout. These karyotypic similarities thus suggest that two separate cutthroat lineages are present in the upper Columbia River system.

The evolutionary affinity suggested between Yellowstone and Great Basin cutthroat trout because of their karyotypic similarities is consistent with past geological and glacial events in the region. Shoshone Falls of the Snake River was formed by lava flows 30 000–60 000 yr ago, isolating the upper Snake River from the rest of the Columbia drainage (Malde 1965). The Bear

River, which is now tributary to Great Salt Lake, was previously a tributary to the Snake River and was diverted into the Bonneville Basin by lava flows (Malde 1965). The transfer of the Bear River to the Bonneville Basin caused the Bonneville Basin to fill up and spill over into the Snake River flood plain ~30 000 yr ago (Bright 1963; in Malde 1965). These geological events could have provided a transfer of cutthroat trout from one system to the other; Hubbs and Miller (1948) proposed that cutthroat trout from the upper Snake River are closely related to Bonneville Basin cutthroat trout.

The Yellowstone Plateau was covered with ice during the latter portion of the Pinedale Glaciation that did not recede until about 7000 yr ago at low altitudes and 6000 at high altitudes (Richmond 1965). Since that time cutthroat trout could have colonized the Yellowstone drainage by migrating from the Snake River over Two Ocean Pass into the headwaters of the Yellowstone River above Yellowstone Lake (Jordan 1891; Behnke 1975).

The west-slope cutthroat trout stock we karyotyped should be representative of this subspecies. Although further studies may detect chromosome polymorphisms within west-slope cutthroat trout, the many subtelocentric chromosomes and higher chromosome number demonstrate karyotypic similarities with coastal cutthroat trout. Historically coastal cutthroat trout inhabited the Columbia drainage as far upstream as Spokane and overlapped the range of west-slope cutthroat trout east of the Cascade Mountains in Washington, where intergrades between the two subspecies were identified (Schultz 1935). Thus, the geographic proximity and chromosome similarities suggest potential evolutionary affinities between these two subspecies. It is possible that west-slope cutthroat trout are the result of an early inland invasion of coastal cutthroat trout and subsequent differentiation in the upper Columbia River system.

This evolutionary scheme is consistent with Behnke's (1973) suggestion that the confusion in the taxonomic status of Columbia River system cutthroat trout is due to postglacial dispersion of two differentiated stocks. It is interesting that these morphologically (Behnke 1973) and karyotypically differentiated subspecies may also be electrophoretically different. Reinitz (1977) characterized electrophoretically the west-slope cutthroat trout and detected malate dehydrogenase, muscle protein, lactate dehydrogenase, phosphoglucomutase, and tetrazolium oxidase polymorphisms, while asparate amino transferase was monomorphic. Loudenslager and Kitchin (1979) characterized electrophoretically the Yellowstone cutthroat trout and detected only an asparate amino transferase polymorphism. Thus a comparison of these accounts suggests that these subspecies may be quite distinct biochemically.

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BEHNKE, R. J. 1965. A systematic study of the family Salmonidae with special reference to the genus *Salmo*. Ph.D. Thesis, University of California, Berkeley, Calif.

1971. The zoogeography, systematics, and management of cutthroat trout. Colorado Coop. Fish. Unit., Colorado State University, Fort Collins, Colo. 8 p.

1973. Rare and endangered species report: west-slope cutthroat trout. Colorado Coop. Fish. Unit., Colorado State University, Fort Collins, Colo. 8 p.

1975. The native cutthroat trout of Wyoming—Part I. Evaluation of collections from the Green River and Bear River drainages; Part II. Further evaluations of collections from the Green River and Bear River drainages. Colorado State University, Fort Collins, Colo. 20 p.

BRIGHT, R. C. 1963. Pleistocene lakes Thatcher and Bonneville, southeastern Idaho. Ph.D. Thesis, University of Minnesota, Minneapolis, Minn.

FUKUOKA, H. 1972a. Chromosome-number variations in the rainbow trout (*Salmo gairdneri irideus* [Gibbons] Jpn. J. Genet. 47: 455–458.

1972b. Chromosomes of the sockeye salmon (*Oncorhynchus nerka*). Jpn. J. Genet. 47: 459–464.

GOLD, J. R. 1974. A fast and easy method for chromosome karyotyping in adult teleosts. Prog. Fish Cult. 36: 169–171.

1977. Systematics of western North American trout (*Salmo*), with notes on the red-band trout of Sheepheaven Creek, California. Can. J. Zool. 55: 1858–1873.

GOLD, J. R., J. C. AVISE, AND G. A. E. GALL. 1977. Chromosome cytology in the cutthroat series, *Salmo clarki* (Salmonidae). Cytologia 42: 377–382.

HECKMAN, J. R., F. W. ALENDORF, AND J. E. WRIGHT. 1971. Trout leukocytes: growth of oxygenated cultures. Science 173: 246–247.

HUBBS, C. L., AND R. R. MILLER. 1948. The zoological evidence; correlation between fish distribution and hydrographic history in the desert basins of western U.S. Univ. Utah Bull. 38(20): 17–166.

JORDAN, D. S. 1891. A reconnaissance of the streams and lakes of the Yellowstone National Park, Wyoming. Bull. U.S. Fish. Comm. IX(1889): 41–63.

LOUDENSLAGER, E. J., AND R. M. KITCHIN. 1979. Genetic similarity between two forms of cutthroat trout, *Salmo clarki*, in Wyoming. Copeia 1979 (4). (In press)

MALDE, H. E. 1965. Snake River plain, p. 255–262. *In* H. E. Wright and D. G. Frey (ed.) The quaternary of the United States. Princeton Univ. Press, Princeton, N.J. 922 p.

MILLER, R. R. 1950. Notes on the cutthroat and rainbow trout with the description of a new species from the Gila River, New Mexico. Occas. Pap. Mus. Zool. Univ. Mich. 529: 1–42.

1972. Classification of the native trouts of Arizona

with the description of a new species, *Salmo apache*. Copeia 1972: 401–422.

MURRAY, J. D., AND R. M. KITCHIN. 1976. Chromosomal variation and heterochromatin polymorphisms in *Peromyscus maniculatus*. Experientia 32: 307–309.

NEEDHAM, P. R., AND R. GARD. 1959. Rainbow trout in Mexico and California with notes on the cutthroat series. Univ. Calif. Publ. Zool. 67: 1–124.

OHNO, S., C. STENIUS, E. FAISST, AND M. T. ZENZES. 1965. Post-zygotic chromosomal rearrangements in rainbow trout (*Salmo irideus*, Gibbons). Cytogenetics 4: 117–129.

PATTON, J. L., AND S. Y. YANG. 1977. Genetic variation in *Thomomys bottae* pocket gophers: Macrogeographic patterns. Evolution 31: 697–720.

REINITZ, G. L. 1977. Electrophoretic distinction of rainbow trout (*Salmo gairdneri*), west-slope cutthroat trout (*S. clarki*), and their hybrids. J. Fish. Res. Board Can. 34: 1236–1239.

RICHMOND, G. M. 1965. Glaciation of the Rocky Mountains. p. 217–232. *In* H. E. Wright and D. G. Frey (ed.) The quaternary of the United States. Princeton Univ. Press. Princeton, N.J. 922 p.

SALAMANCA, F., AND S. ARMENDARES. 1974. C-bands in human metaphase chromosomes treated with barium hydroxide. Ann. Genet. 17: 135–136.

SCHULTZ, L. P. 1935. Species of salmon and trout in the northwestern United States. Proc. Fifth Pac. Sci. Cong. 5: 3777–3782.

SIMON, R. C. 1964. Cytogenetics, relationships, and evolution in Salmonidae. Ph.D. Thesis, University of Washington, Seattle, Wash.

SIMON, R. C., AND A. DOLLAR. 1963. Cytological aspects of speciation in two North American Teleosts, *Salmo gairdneri* and *Salmo clarki lewisi*. Can. J. Genet. Cytol. 5: 43–49.

THORGAARD, G. H. 1976. Robertsonian polymorphism and constitutive heterochromatin distribution in chromosomes of the rainbow trout (*Salmo gairdneri*). Cytogenet. Cell Genet. 17: 174–184.

1978. Sex chromosomes in the sockeye salmon: a Y-autosome fusion. Can. J. Genet. Cytol. 20: 349–354.

WILMOT, R. L. 1974. A genetic study of the red-band trout. Ph.D. Thesis, Oregon State University, Corvallis, Oreg.

Plaque-Forming Cells and Humoral Antibody in Rainbow Trout (*Salmo gairdneri*) Induced by Immersion in a *Yersinia ruckeri* O-Antigen Preparation

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Rainbow trout (*Salmo gairdneri*) were exposed to the O-antigen of *Yersinia ruckeri* by various immunization regimens. The passive hemolytic plaque assay was used to show specific splenic plaque-forming cells (PFC) and passive hemagglutination demonstrated humoral antibody titers in fish injected with or immersed in the antigen preparations. Preceding antigen immersion with a 2-min immersion in either a 5.2 or a 2.6% NaCl solution did not affect the numbers of PFC or levels of antibody. Fish held for 2 min in ethyl *m*-aminobenzoate methanesulfonate (MS-222®) before antigen immersion showed no significant difference from the control fish. Dosage studies showed that the minimal concentration of antigen for induction of PFC by immersion was 5.0 μg/mL.

Key words: plaque-forming cells, immunization, rainbow trout immersion, *Yersinia ruckeri*

ANDERSON, D. P., B. S. ROBERSON, AND O. W. DIXON. 1979. Plaque-forming cells and humoral antibody in rainbow trout (*Salmo gairdneri*) induced by immersion in a *Yersinia ruckeri* O-antigen preparation. J. Fish. Res. Board Can. 36: 636–639.

Des truites arc-en-ciel (*Salmo gairdneri*) furent exposées à l'antigène-O de *Yersinia ruckeri* selon divers régimes d'immunisation. L'essai hématolytique passif des plaquettes a été utilisé pour montrer la présence de cellules de la rate formant des plaquettes (FPC). L'hémoagglutination passive servit à démontrer les titres d'anticorps humoraux chez des poissons auxquels on avait injecté des préparations d'antigène ou dans lesquels on les avait immergés. Avant immersion dans l'antigène, une immersion de 2 min dans une solution de NaCl soit à 5,2%, soit à 2,6% n'affecte pas le nombre de PFC ou les niveaux d'anticorps. Les poissons maintenus durant 2 min dans l'anésthésique métasulfonate de tricaïne (MS-222®) avant immersion dans l'antigène ne diffèrent pas sensiblement des poissons témoins. Des dosages démontrent que la concentration minimale d'antigène requise pour produire des PFC par immersion est de 5,0 μg/mL.

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MASS immunization of fish by various methods of exposure to antigens is an area of intensive research in fish culture (Fryer et al. 1977). Present methods of administering vaccine to fishes include injection routes, oral immunization (Klontz and Anderson 1970), hyperosmotic immersion (Antipa and Amend 1977; Croy and Amend 1977), and spraying (Gould et al. 1978).

The detection of antibody after immunization helps to confirm specific sensitization; however, the presence of antibody is not necessarily correlated with protection in the field (Gunnels et al. 1976). Croy and Amend (1977) showed that protection against *Vibrio anguillarum* occurred when coho salmon (*Oncorhynchus kisutch*) were immunized by immersion and later challenged with the virulent pathogen by water contact; however, antibody titers among test and control fish did not appear to differ significantly. Anderson and Nelson (1974) compared the protection provided to

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rainbow trout (*Salmo gairdneri*) by injection with that provided when the fish were fed chloroform-killed *Yersinia ruckeri* bacterins. Protection was demonstrated in both groups, but no antibody was found in the fish fed the bacterin.

The passive hemolytic plaque assay has recently been adapted for the detection of specific plaque–forming cells (PFC) in rainbow trout that have been injected with an O-antigen of *Y. ruckeri* (Anderson et al. 1979). Upon incubation with the labeled sheep red blood cells (SRBC), the PFC producing the specific antibody are surrounded by hemolytic lysis, thus giving a sensitive indication of the immune response.

This paper reports experiments on the immune response of rainbow trout exposed to a *Y. ruckeri* O-antigen preparation following different regimens of antigenic exposure: hyperosmotic infiltration, parenteral injection, or simple immersion. In addition, the effect on the immune response of an anesthetic treatment before exposure to antigen by immersion was tested, and a dosage study was done by simple immersion of the fish in various dilutions of antigen preparations.

Materials and Methods

Rainbow trout 300–400 g each, obtained from the Experimental Hatchery, Leetown, West Virginia, were held in either heated (17 ± 1.5°C) or unheated (13 ± 1.0°C) spring water flowing through concrete or fiberglass troughs. The trout were fed a commercial pelletized dry feed (no. PR1130, Zeigler Bros. Inc., Gardners, Pa.).

The O-antigen of an isolate of *Y. ruckeri* (no. 11.29 from the permanent file of the Bacteriology Section of the National Fish Health Research Laboratory, Leetown, West Virginia) was prepared by an acetone–alcohol method developed by Edwards and Ewing (1972). The dried, powdered antigen was boiled and cooled before injection into fish or absorption on sheep red blood cells (SRBC). Antigen concentrations are reported on the basis of dry weight.

Methods of exposing the fish to the antigen were compared by dividing 25 fish into five equal groups and subjecting each group to a different immunization regimen. Fish in group no. 1 were injected intraperitoneally with 1 mL of phosphate-buffered saline (PBS), pH 7.2, containing 100 μg of *Y. ruckeri* O-antigen. Fish in group no. 2 were held in a 2.6% NaCl solution for 2 min, then transferred and held for 2 min in the antigen preparation containing 100 μg/mL in spring water. Group no. 3 fish were held in a 5.2% NaCl solution for 2 min, then transferred to an antigen preparation of 100 μg/mL in spring water for 2 min. Group no. 4 fish were held for 2 min in the antigen preparation of 100 μg/mL in spring water. Group no. 5 fish were held in a 2.6% NaCl solution for 2 min. All fish were marked by cold branding and held as one lot in a concrete raceway for 14 d at 17 ± 1.5°C before sampling.

In the immersion experiments, fish were individually dipped into the antigen preparations. Because of the difficulty of handling the active fish during this procedure, we tested the effects of mild anesthesia on the immune response. Five fish were anesthesized with ethyl m-aminobenzoate methanesulfonate (MS-222®), 1:20 000 wt/vol for 2 min before being placed in the antigen bath. After anesthetization the fish were placed in the antigen preparation (100 μg/mL in spring water) for 2 min, then they were held in individual troughs at 13 ± 1.0°C for 14 d before samples were taken.

Six groups of five fish each were exposed by simple immersion with dilutions of the O-antigen of *Y. ruckeri*. Trout were placed, without anesthesia, in the appropriate antigen preparations at 100-, 50-, 5-μg, 500-, 50-, or 5 ng/mL for 2 min, then transferred to a holding container for 5 min to ensure rinsing of the antigen before they were returned to the trough. Fish were held at 13 ± 1.0°C in individual fiberglass troughs for 16 d before sampling.

The passive hemolytic plaque assay, an adaptation of the Jerne plaque assay (Jerne and Nordin 1963) was used to detect leukocytes producing antibody against the specific O-antigen. The method for labeling the SRBC was described by Anderson et al. (1979). Washed SRBC were incubated with an equal volume of *Y. ruckeri* O-antigen (1 mg/mL) in PBS pH 7.2 for 1 h at 37°C. The suspension was placed in the refrigerator overnight before being washed 3 times in PBS pH 7.2 the next day in preparation for the assay. Passive hemagglutination was used to detect humoral antibody. The same labeled SRBC used for the passive hemolytic plaque assay were used as the antigen in the microtiter tests of passive hemagglutination.

Statistical comparisons were obtained between and among groups of fish by analysis of variance.

Results

Trout placed in either the 5.2 or the 2.6% NaCl solutions before immersion in the 100 μg/mL antigen preparation had approximately the same numbers of PFC and antibody titers as the trout immersed in the antigen without pretreatment or the trout given the intraperitoneal injection of 100 μg of the antigen. No specific PFC or humoral antibody titers were observed in the control fish dipped in the 2.6% solution (Table 1). No statistical differences were demonstrated by a two-way analysis of variance among the test fish groups exposed by different regimens to the antigen preparation.

Placing the trout in an MS-222® solution before immersion into the O-antigen preparation had little effect on the numbers of PFC when compared with the numbers in concurrent control trout that were exposed by antigen immersion without anesthetic (Table 2). No humoral antibody was detected in fish held at 13 ± 1.0°C and sampled 14 d after antigen exposure. There were no statistical differences between groups of exposed fish by a one-way classification analysis of variance.

Trout responded with PFC and humoral antibody after immersion in antigen concentrations of 5.0 μg/mL or more for 2 min. These fish were held for 16 d after antigen exposure at 13 ± 1.0°C (Table 3). The appearance of humoral antibody generally corresponded to the appearance of PFC in the individual fish sampled.

Discussion

Specific plaque-forming cells and humoral antibody

TABLE 1. Comparisons of numbers of PFC and antibody in each of five rainbow trout exposed to *Yersinia ruckeri* O-antigen by different methods: A) injected with 100 μg antigen suspended in 1 mL PBS, B) immersed in a 2.6% NaCl solution then in the antigen preparation (concentration 100 μg/mL), C) immersed in a 5.2% NaCl solution then in the antigen preparation, D) immersed only in antigen preparation, E) immersed only in a 2.6% NaCl solution. The fish were held at 17 ± 1.5°C for 14 d before sampling. Each value for an individual fish is given as PFC/10^6 lymphocytes followed by the corresponding humoral antibody titer in parentheses.

	Treatment				
	A	B	C	D	E
	100 (128)	0 (64)	0 (128)	0 (0)	0 (0)
	0 (0)	0 (0)	200 (128)	30 (128)	0 (0)
	no test	0 (0)	20 (64)	120 (256)	0 (0)
	60 (512)	0 (64)	80 (1024)	160 (256)	0 (0)
	40 (64)	200 (1024)	130 (2048)	250 (32)	0 (0)
Mean ± SD	50 ± 42 (45)	40 ± 90 (21)	86 ± 82 (294)	112 ± 101 (48)	0 (0)

were induced in rainbow trout given *Y. ruckeri* O-antigen by injection, hyperosmotic infiltration, or by simple immersion into the antigen preparations. The passive hemolytic plaque assay enabled us to detect the immune response in the trout 14 d after exposure to the antigen, even though the fish were held in temperatures that are known to delay the formation or release of antibody.

TABLE 2. Effect of pretreatment with MS-222® compared with no treatment before antigen immersion on numbers of splenic PFC and humoral antibody titer. Fish were held for 14 d at 13 ± 1.0°C before sampling. Results are on individual fish and are expressed as PFC/10^6 lymphocytes. No humoral antibody was detected in these fish.

Anesthetic pretreatment	Antigen immersion alone
0	30
100	10
60	300
200	400
50	1400
Mean ± SD 82 ± 74	428 ± 569

Antipa and Amend (1977) reported antibody in the sera of coho salmon (*Oncorhynchus kisutch*) at 5 wk after a hyperosmotic immersion treatment with either a suspension of *Vibrio anguillarum*, *Aeromonas salmonicida*, or a combination of the vaccines. Their technique of antibody detection was by agglutination of formalin-killed bacteria. From the results of our studies, it appeared that an immersion in a salt solution before immersion in the *Y. ruckeri* O-antigen solution was not necessary for the stimulation of PFC and humoral antibody. Indeed fish placed 2 min in a 5.2% NaCl bath before immersion in the antigen preparation did not give significantly higher numbers of PFC or titers of humoral antibody. In fact, the fish held in 2.6% NaCl solution before the antigen dip developed fewer PFC and lower antibody titers than did the other groups of fish exposed to the antigen. Although it is remarkable that the trout exposed by the simple immersion method seemed to give high numbers of PFC and antibody titers compared with the injected fish, the actual preferred route of immunization may still remain in question because the difference among the groups was not critically significant. In addition, the mechanisms of antigen uptake may influence the time–dose–

TABLE 3. Splenic PFC lymphocytes and corresponding antibody titers of each of five rainbow trout immersed in selected doses of *Yersinia ruckeri* O-antigen for 2 min and held at 13 ± 1.0°C for 16 d after antigen exposure. Each value for an individual fish is given as PFC/10^3 lymphocytes followed by the humoral antibody titer in parentheses.

	Exposure dose/mL[a]			
	100 μg	50 μg	5 μg	500 ng
	1.8 (16)	11.4 (64)	1.7 (4)	0 (0)
	0 (16)	0.5 (0)	0 (8)	0 (0)
	3.2 (64)	0 (0)	0.6 (2)	0 (4)
	2.0 (64)	0.7 (0)	0 (2)	0 (0)
	3.4 (128)	0.8 (32)	0 (4)	0 (0)
Mean ± SD	2.1 ± 1.3 (42)	2.7 ± 4.9 (5)	0.5 ± 0.8 (4)	0 (<2)

[a]No PFC or humoral antibody detected at 50- or 5-ng doses.

response curves for the maximum numbers of PFC, humoral antibody, and protection. For instance, if the injected antigen were to gain access more rapidly to initiate the processes of an immune response than the antigens given by immersion techniques, different kinetics of the immune response may be observed.

Since difficulties of uniform exposure were encountered when the trout had to be handled individually for immersion into the antigen preparations, experiments with an anesthetic pretreatment were done. Results showed that when the trout were held for 2 min in a 1:20 000 dilution of MS-222® before antigen exposure, they appeared to develop fewer PFC than did the control immunized fish. Although the comparison was not statistically significant, this information may be helpful for future immunization experiments where the fish have to remain calm to ensure better calibration of antigen dose.

From the data gathered in the dosage studies, it was interesting to observe not only the sensitivity of the fish to low doses of antigen when administered by the immersion method, but also to compare these results with a previous dosage study done with the same antigen given by an intraperitoneal injection (Anderson et al. 1979). Fish responded to immersion doses of 5 μg/mL and above, whereas the fish injected with the antigen were sensitive to 0.5 μg. Although the actual antigen dose that the fish receives by total immersion would be difficult to evaluate, the doses needed in both situations were remarkably low.

The holding temperatures influenced the later detection of PFC and antibody in our experiments. Because the heated water environment was expensive to maintain, not all of our tests could be carried out at 17 ± 1.5°C. In the tests completed, humoral antibody was apparent at the time of sampling (14 d after antigen exposure) in the fish held at 17 ± 1.5°C, but not in fish held at 13 ± 1.0°C. However, by 16 d after antigen exposure lower titers of antibody were found in fish held at the lower temperatures during the dosage studies. In earlier studies it was found that the optimal numbers of PFC occurred at day 11 after injection when the fish were held at 17 ± 1.5°C, and about 16 d after injection when the fish were held at 13 ± 1.0°C (Anderson et al. 1979).

Many questions remain about the physiological mechanisms involved in the absorption or uptake of the antigen by the trout immersed in a vaccine. Amend and Fender (1976) indicated that bovine serum albumin was taken into the fish by the lateral line and the gill areas through the pressures of hyperosmotic infiltration, although their later studies (Fender and Amend 1978) using epinephrine showed that the gill may not be the main organ of absorption. Our work demonstrates that the uptake of antigen does not depend upon the salt treatment but may itself be an active process. Future areas of research on the initial steps of the stimulation of the immune response in salmonids may include active selection, surface absorption, and macrophage uptake of antigen.

AMEND, D. F., AND D. C. FENDER. 1976. Uptake of bovine serum albumin by rainbow trout from hyperosmotic solutions: A model for vaccinating fish. Science 192: 793–794.

ANDERSON, D. P., AND J. R. NELSON. 1974. Comparison of protection in rainbow trout (*Salmo gairdneri*) inoculated with and fed Hagerman redmouth bacterins. J. Fish. Res. Board Can. 31: 214–216.

ANDERSON, D. P., B. S. ROBERSON, AND O. W. DIXON. 1979. Cellular immune response in rainbow trout (*Salmo gairdneri*) to *Yersinia ruckeri* O-antigen monitored by the passive hemolytic plaque assay. J. Fish Dis. (In press)

ANTIPA, R., AND D. F. AMEND. 1977. Immunization of Pacific salmon: Comparison of intraperitoneal injection and hyperosmotic infiltration of *Vibrio anguillarum* and *Aeromonas salmonicida* bacterins. J. Fish. Res. Board Can. 34: 203–208.

CROY, T. R., AND D. F. AMEND. 1977. Immunization of sockeye salmon (*Oncorhynchus nerka*) against vibriosis using hyperosmotic infiltration. Aquaculture 12: 317–325.

EDWARDS, P. R., AND W. H. EWING. 1972. Identification of Enterobacteriaceae. Burgess Publishing Company, Minneapolis, Minn. 362 p.

FENDER, D. C., AND D. F. AMEND. 1978. Hyperosmotic infiltration: factors influencing uptake of bovine serum albumin by rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 35: 871–874.

FRYER, J. L., D. F. AMEND, L. W. HARRELL, A. J. NOVOTNY, J. A. PLUMB, J. S. ROHOVEC, AND G. L. TEBBIT. 1977. Development of bacterins and vaccines for control of infectious diseases in fish. Oreg. State Univ. Sea Grant Coll. Prog. Publ. No. ORESU-T-77-012: 8 p.

GOULD, R. W., P. J. O'LEARY, R. L. GARRISON, J. S. ROHOVEC, AND J. L. FRYER. 1978. Spray vaccination: A method for the immunization of fish. Fish Pathol. 13: 63–68.

GUNNELS, R. D., H. O. HODGINS, AND M. H. SCHIEWE. 1976. Failure of vaccines to protect salmon from *Vibriosis* enzootic in Puget Sound, Washington. Am. J. Vet. Res. 37: 737–740.

JERNE, N. K., AND A. A. NORDIN. 1963. Plaque formation in agar by single antibody-producing cells. Science 140: 405.

KLONTZ, G. W., AND D. P. ANDERSON. 1970. Oral immunization of salmonids: A review, p. 16–20. *In* S. F. Snieszko [ed.] A symposium on diseases of fishes and shellfishes. Am. Fish. Soc. Spec. Publ. No. 5.

Depression of pH in Lakes and Streams in Central Ontario During Snowmelt

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JEFFRIES, D. S., C. M. COX, AND P. J. DILLON. 1979. Depression of pH in lakes and streams in central Ontario during snowmelt. J. Fish. Res. Board Can. 36: 640–646.

The snow pack that accumulated in central Ontario in the winter of 1977–78 had a pH of 4.0–4.5. The resultant runoff in the following spring in three intensively studied watersheds was characterized by a 2–13-fold increase in H^+ content. Between 36 and 77% of the year's export of H^+ from the watersheds occurred in April. Similar pH depressions were observed in almost all of 17 other streams that were less frequently sampled and in the littoral zones, surface waters, and outflows of five lakes.

Key words: acidification, snowmelt, hydrogen ion

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La couche de neige qui s'était accumulée en Ontario central durant l'hiver 1977–78 avait un pH de 4,0–4,5. Le ruissellement qui en est résulté au printemps suivant dans trois bassins hydrographiques intensivement étudiés accusait une augmentation de 2 à 13 fois de la teneur en H^+. De 36 à 77% de l'exportation de H^+ pour l'année en provenance de ces bassins se produisit en avril. On observe des diminutions semblables de pH dans presque tous les 17 autres cours d'eau échantillonnés moins fréquemment et dans les zones littorales, les eaux superficielles et les émissaires de cinq lacs.

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DEPRESSION of pH in streams during spring runoff has been observed in Finland (Haapla et al. 1975), Sweden (Odén and Ahl 1970; Hultberg 1976), and Norway (Gjessing et al. 1976; Henriksen and Wright 1977). This effect has been attributed in all cases to the rapid melt of an accumulated snowpack of low pH and occurs in areas underlain by Precambrian bedrock that have overburden incapable of buffering the runoff waters. The pH's of accumulated snow in these areas are typically 4.0–4.5 (Hultberg 1976), and this may, in fact, result in runoff that is more acidic than the snow itself. Johannessen et al. (1977) reported that in laboratory studies, the first 30% of the meltwater from a snow sample contained 70–80% of the H^+, $SO_4^=$, and NO_3^-. This meltwater normally forms a surface layer of several meters thickness which may extend over the entire surface of the lake into which it drains. For example, Hultberg (1976) observed a drop in pH from 5.0 to 4.2 in the surface waters of Lake Timmervatten, Sweden, and from 5.0 to 3.4 in Lake Stensjon, while Dickson (1975) reported that pH declined from 4.9 to 4.5 in the mountain lake Övre Särnamannasjön which had an accumulation of snow of pH 4.2 in its watershed. The top 2 m of Langtjern, a small lake in south–central Norway, dropped 0.4–0.5 pH units in all years of study (Henriksen and Wright 1977) while the upper layer in Sandtjern dropped almost 2.0 pH units (Gröterud 1972) during spring runoff. Similar findings have been reported for lakes in the LaCloche Mountain area near Sudbury, Ontario, by Beamish (1974) and Yan and Stokes (1978).

Reduction in the pH of lake tributaries, surface waters, and littoral zones during the spring snowmelt period can have deleterious effects on the biota inhabiting these areas (Bell 1971; Muniz et al. 1975; Börgström and Hendrey 1976). In south–central Ontario the fish populations are of special interest. Those species that have acid-sensitive life stages coinciding with snowmelt may be particularly susceptible. Spawning, fry hatch, and early development have previously been identified as critically sensitive stages (Almer et al. 1978). Species endemic to the region and spawning during the April–May snowmelt period include northern pike (*Esox lucius*), yellow perch (*Perca flavescens*), walleye (*Stizostedion vitreum vitreum*), and introduced rainbow trout (*Salmo gairdneri*) (Scott and Crossman 1973). Lake trout (*Salvelinus namaycush*), brook trout (*S. fontinalis*), lake whitefish (*Coregonus clupeaformis*), and cisco (*C. artedii*) spawn during the fall with egg hatch and early fry development sometimes coinciding with spring snowmelt. Many of these potentially susceptible species also make up a significant portion of the sport fishery in south–central Ontario. Because melt

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TABLE 1. Description of three watersheds; drainage area (A_d) in square kilometres. Chemical values are averages of 30–40 measurements excluding the spring runoff period. Color in Hazen Units, alkalinity in meq·L^{-1}.

Watershed	General morphology	Geology		pH	Alk.	Color
		Bedrock	Surficial			
Paint Lake no. 1 45°13′N, 78°57′W (A_d = 0.21 km²)	Steeply sloping watershed (slope 10–20%); stream drains a small centrally located pond covering ≃5% of the total drainage area.	Biotite gneiss with minor occurrence of pegmatite dykes.	Unsorted basal till, generally <1 m thick. Exposed bedrock common, particularly in stream.	5.7	0.07	5
Harp Lake no. 4 45°23′N, 79°08′W (A_d = 1.24 km²)	Variable slope (4–5% avg) containing small centrally located pond covering ≃2% of the total drainage area.	Amphibolite and schist; ~15% is underlain by biotite gneiss.	Unsorted till (≃1 m) predominates with significant occurrence of exposed bedrock and a clean well-sorted sand.	6.2	0.18	40
Dickie Lake no. 10 45°23′N, 79°05′W (A_d = 0.66 km²)	Flat lying, avg slope = 1–2%.	Horneblende migmatite.	Thin till and exposed bedrock predominates on the periphery while a sequence (≃1 m) of peat over clay over sand is found in the center.	4.7	0.05	200

of the snowpack that typically has a pH of 4.0–4.5 in this area (Scheider et al. 1979) may result in deleterious effects on these fisheries, we have investigated the water chemistry of several streams and the littoral zones of several lakes in this susceptible area.

Study Area

A general description of the study area has been given by Dillon et al. (1978a). Three small streams were chosen for intensive monitoring throughout the spring runoff period. A description of the morphology, bedrock, and surficial geology is given for each in Table 1, as well as average pH, alkalinity, and color for 1977. All the streams drain small watersheds and have peak discharges of <200 L·s^{-1}. The streams and watersheds of each lake were arbitrarily numbered for identification. Harp Lake Inflow no. 4 and Paint Lake Inflow no. 1 are steeply sloping watersheds, each stream draining a small pond. The Dickie Lake Inflow no. 10 watershed is relatively flat (1–2% mean slope) with surficial deposits in the center of the basin consisting of a sequence of peat overlaying an impermeable layer of clay up to 15 cm thick which, in turn, covers a layer of clean, well-sorted sand of variable thickness resting on bedrock. Drainage from the basin periphery passes over exposed bedrock and thin till before entering the peat layer where it obtains its high organic content and dark brown color.

The average pH values reflect the degree of contact of precipitation with the lithographic bases in the watershed. Contact is minimal in the Dickie Lake no. 10 basin where the impermeable clay layer controls the drainage flow. Relatively more contact is experienced in the Harp Lake no. 4 and Paint Lake no. 1 basins with the difference in average pH between the two attributable to the shorter relative residence time of runoff within the smaller, steeper Paint Lake basin.

In addition to these three streams, 14 other streams flowing into four of the study lakes, these four lakes' outflows, and three larger rivers (Black River, Oxtongue River, and Maple Creek) all in the same area were studied in the spring of 1978.

Materials and Methods

Water samples for chemical analysis were collected from Harp Lake Inflow no. 4, Dickie Lake Inflow no. 10, and Paint Lake Inflow no. 1 throughout most of April 1978 using either SISCO or ISCO automatic samplers that withdrew water from the pool formed behind the weir of each stream at 1–3-h intervals for about 20 d. The intakes of the samplers were positioned to prevent contamination from stream sediment or floating debris. Discharge was calculated from measurements of water velocity using either Ott C31-00 or Teledyne Gurley Pygmy model 625 current meters, taken across a surveyed section of each stream.

Grab samples were collected twice weekly from 14 other inflows and four outflows in our study area and on a daily basis from the three larger rivers noted above. Samples for chemical analysis were collected from the littoral zones of five lakes (Harp, Dickie, Chub, Crosson, and Fawn) in the study area. All were sampled at 1 m depth at three locations or more using a Van Dorn bottle.

Bulk precipitation was collected by a network of samplers described by Dillon et al. (1978a). Rain samples were collected with 26.7-cm diam funnels leading into glass bottles. Funnels were fitted with 500-μm Nitex mesh to prevent contamination by insects. Snow samples were collected with 63-cm high, 43-cm diam polyethylene containers. Rainfall depths were measured at each collector site using 10-cm diam polycarbonate rain gauges. Snow water equivalent depths were obtained from melted snow samples. Daily loading rates (total deposition per collection period/number of days in the period) were calculated for each collector and a mean daily load was obtained and summed to give an annual load from June 1977 to May 1978.

TABLE 2. Monthly discharge and hydrogen ion loads and percent of annual total for Paint Lake no. 1, Harp Lake no. 4, and Dickie Lake no. 10 from June 1977 through May 1978.

	Paint Lake no. 1				Harp Lake no. 4				Dickie Lake no. 10			
	Discharge		H^+ Load		Discharge		H^+ Load		Discharge		H^+ Load	
	$10^3 m^3$	%	Equiv.	%	$10^3 m^3$	%	Equiv.	%	$10^3 m^3$	%	Equiv.	%
June 1977	0.27	0.2	0.53	0.1	6.20	1.0	2.98	0.4	0.00	0.0	0	0.0
July	0.15	0.1	0.24	0.1	2.70	0.4	0.59	0.1	0.00	0.0	0	0.0
Aug.	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0	0.0
Sept.	0.81	0.7	0.72	0.2	25.6	4.2	5.86	0.8	26.9	7.4	599	7.5
Oct.	10.9	9.3	16.7	3.4	61.1	10.0	26.6	3.6	76.4	21.1	2050	25.8
Nov.	15.5	13.2	49.1	10.0	77.1	12.6	40.9	5.5	38.2	10.6	789	9.9
Dec.	6.70	5.7	21.2	4.3	33.2	5.4	21.7	2.9	30.8	8.5	694	8.7
Jan. 1978	3.35	2.9	7.95	1.6	21.0	3.4	9.17	1.2	11.4	3.2	139	1.8
Feb.	1.81	1.5	5.74	1.2	12.5	2.0	5.31	0.7	5.40	1.5	86	1.1
Mar.	3.85	3.3	11.4	2.3	14.8	2.4	4.53	0.6	9.80	2.7	134	1.7
Apr.	53.2	45.3	298	60.8	282	46.1	566	76.6	129	35.6	2870	36.2
May	20.9	17.8	78.9	16.1	75.7	12.4	55.4	7.5	34.1	9.4	575	7.5
Total	117		490		612		740		360		7940	

Chemical analyses of water samples were completed as soon as possible after sampling, always within 24 h. A Corning Digital 109 pH meter was used to measure pH while alkalinity was determined by titration with 0.02 N H_2SO_4 (0.01 mol·L^{-1}) to an endpoint of pH = 4.50. As discussed by Dillon et al. (1978a), this "total fixed-endpoint alkalinity" overestimates the true acid-neutralizing potential of water of dilute ionic composition, and thus the reported values should be considered upper limits.

Analytical techniques are reported in detail in Ontario Ministry of the Environment (1975).

Results and Discussion

Discharge, pH, and hydrogen ion load for Paint Lake no. 1, Harp Lake no. 4, and Dickie Lake no. 10 are shown in Fig. 1a–c for the period March 25–May 15, 1978. The occurrence and magnitude of precipitation events are also presented. Precipitation throughout April was very light and hence had little effect on the stream discharges and calculated loadings. In all three cases, stream pH declined as discharge increased, reached a minimum at peak discharge, and increased again as pH returned to pre-runoff levels. The decline was 0.5, 1.2, and 0.3 units in Paint Lake no. 1, Harp Lake no. 4, and Dickie Lake no. 10, respectively, corresponding to increases in H^+ content from 3 to 10 μeq·L^{-1}, 0.3 to 4 μeq·L^{-1}, and 16 to 32 μeq·L^{-1}.

The observation of Johannesen et al. (1977) and Johannessen and Henriksen (1977) that the initial portion of the snowmelt contains a proportionately large percentage of the ions in the snow including H^+ is not supported by our data. The first 33% of the runoff contributed 32, 21, and 39% of the H^+ load in Paint Lake no. 1, Harp Lake no. 4, and Dickie Lake no. 10, respectively. The hydrographs and plots of H^+ load are very similar in shape for all three streams.

The importance of the spring period in the annual hydrologic budget of north-temperate watersheds is well known (e.g. Likens et al. 1977; Schindler et al. 1976); the importance of this period with regard to the annual H^+ budget is demonstrated in Table 2. In April 1978, the month during which almost all of the snowmelt runoff occurred, 61, 77, and 36% of the year's export of H^+ from the Paint Lake no. 1, Harp Lake no. 4, and Dickie Lake no. 10 watersheds occurred along with 45, 46, and 36% of the annual discharge. The proportionately greater input of H^+ in Paint Lake no. 1 and Harp Lake no. 4 is, of course, consistent with the lowered pH at this time of year.

During June 1977–May 1978 we measured the input of H^+ from the atmosphere to be 71 meq·m^{-2}. The output or export of H^+ from the three watersheds was 2.3, 0.55, and 13.5 meq·m^{-2} for Paint Lake no. 1, Harp Lake no. 4, and Dickie Lake no. 10, respectively; hence the retention of H^+ in the watersheds or neutralization in the streams or watersheds was 97, 99, and 81%. Therefore, all three have considerable buffering capability (or cation exchange capacity) remaining, particularly the Paint Lake and Harp Lake watersheds; however, during spring runoff and probably during major storm events the buffering reactions are not fast enough to prevent a decline in stream pH (Scheider et al. 1979).

The cations in the podzolic overburden replaced by H^+ include calcium, magnesium, aluminum, and to a lesser extent manganese. Elevated aluminum and manganese levels in many lakes in the study area have been reported by Scheider et al. (1979); the possibility that calcium and magnesium concentrations are also elevated is harder to evaluate. Levels in the lakes and streams in the area are low but variable (e.g. 2–10 mg Ca^{++}·L^{-1}) and probably dependent on the exact nature

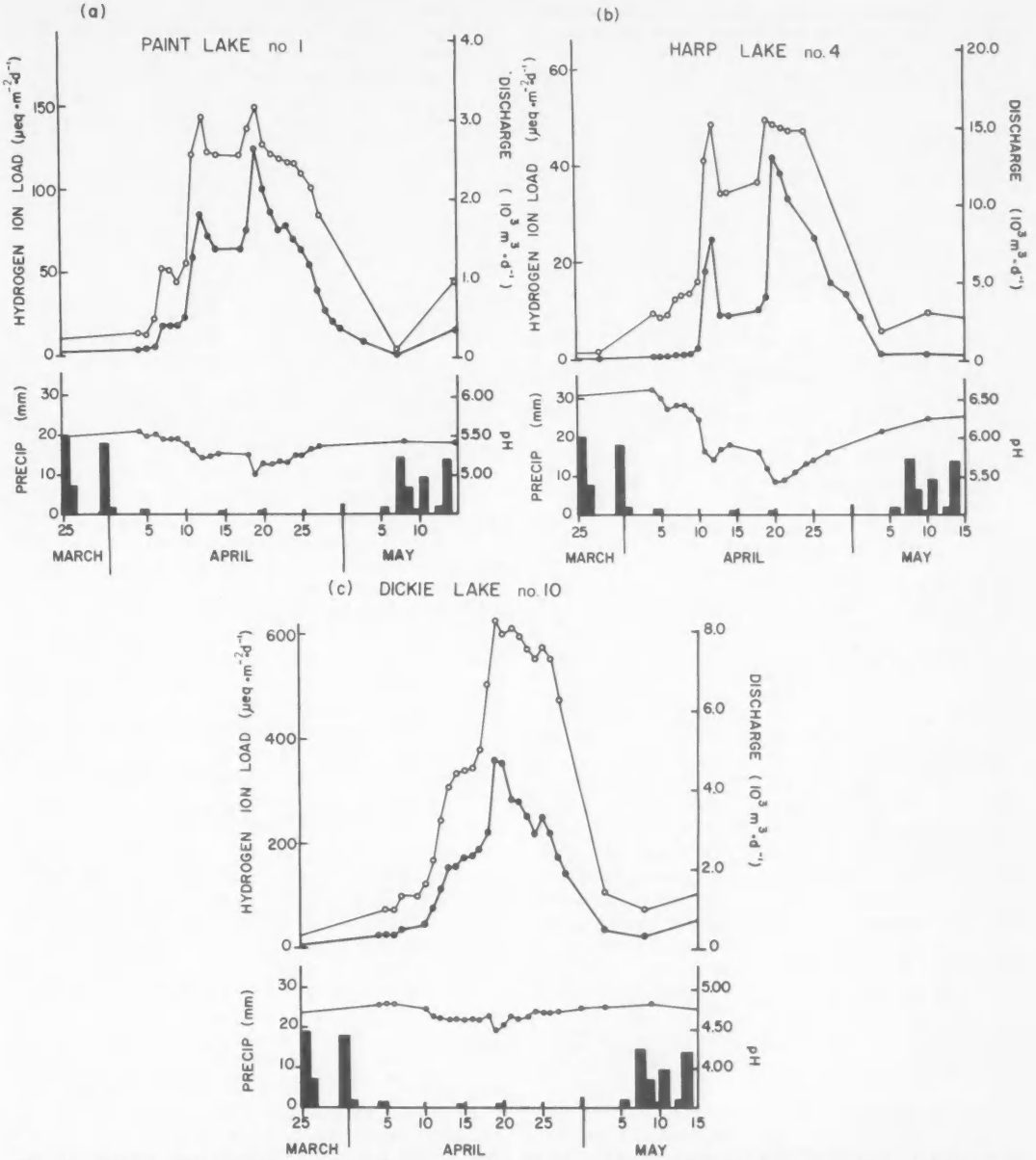

FIG. 1. Discharge (○), hydrogen ion load per unit area of each watershed (●), pH (lower graph), and depth of precipitation for each day that a precipitation event occurred for (a) Paint Lake no. 1, (b) Harp Lake no. 4, and (c) Dickie Lake no. 10. Daily H^+ load to the respective lakes can be calculated by multiplying by the watershed area: lake area (A_d/A_o); where A_d is the area of the subwatershed and A_o that of the ake; A_d/A_o is 1.85 for Harp Lake no. 4, 0.71 for Dickie Lake no. 10, and 0.14 for Paint Lake no. 1.

and thickness of the thin overburden in individual watersheds. Because the runoff component of the hydrologic cycle is $\simeq 0.5\ m^3 \cdot m^{-2} \cdot yr^{-1}$ in this part of Ontario (i.e. mean precipitation–evapotranspiration on an areal basis (Pentland 1968)), complete replacement of H^+ and Ca^{++} would result in an increase in Ca^{++} con-

TABLE 3. pH of streams in Muskoka–Haliburton; stream pH is given prior to spring runoff (mid-March 1978) and at maximum runoff (mid-April 1978).

Watershed	Stream	pH	
		Mid-March	Mid-April
Harp Lake	3	6.1	5.1
	3A	6.0	5.6
	5	5.9	4.8
	6	6.2	5.3
	6A	5.4	5.0
	Outflow	6.3	5.0
Dickie Lake	5	4.6	4.3
	6	4.6	4.4
	11	4.9	4.1
	Outflow	5.6	4.9
Chub Lake	1	5.8	5.1
	2	5.2	4.7
	Outflow	5.5	4.8
Red Chalk Lake	1	6.1	5.6
	2	4.5	4.3
	3	6.0	5.5
	4	6.2	5.5
	Outflow	6.1	5.9
Maple Lake	Maple Creek	6.2	5.8
Lake Simcoe	Black River (at Vankoughnet)	6.3	5.9
Lake of Bays	Oxtongue River	6.3	6.1

centration in streams of 2–3 mg $Ca^{++} \cdot L^{-1}$, a change that is within the natural variation found in the area.

The pH of other streams in the study area was also monitored in the spring of 1978. These included many unnamed streams draining small watersheds as well as a few large rivers. The pH in mid-March before spring runoff began is compared with that measured at peak runoff in Table 3. In all cases, an increase in H^+ concentration at peak runoff was observed. As expected, the small streams generally had greater changes, especially in terms of increase in H^+ concentration. However, even in large rivers such as the Black and Oxtongue, the pH declined. These minor pH depressions which ranged from 0.2 to 0.4 pH units were accompanied by reduction in alkalinity, from, for example, $\simeq$0.16 to $\simeq$0.06 meq $\cdot L^{-1}$ in the Black River and from $\simeq$0.18 to $\simeq$0.06 meq $\cdot L^{-1}$ in the Oxtongue River.

Because meltwater which is at or near 0°C is less dense than lakewater at 4°C, it forms an acidic surface layer that may cover the entire lake including the littoral zone to a depth of several meters and may in part be passed through the outflow. The pH's of the outflows of Harp, Dickie, Chub and Red Chalk Lake, shown in Table 3, were all reduced in April compared with May, probably as a result of the movement of acidic water across the top of each lake. The pH's of near-surface waters (1 m) of several lakes in April 1978 are shown in Table 4. These samples included a major proportion

TABLE 4. Average pH at 1 m during spring runoff (April 1978) and for whole lake in February for lakes in Muskoka–Haliburton. *n*, number of sample locations at each date.

Lake	Date sampled	Average pH (*n*)	February composite pH
Harp Lake	Apr. 12/78	6.2 (2)	6.1
	Apr. 19/78	5.4 (3)	
	Apr. 27/78	5.7 (4)	
Dickie Lake	Apr. 26/78	4.8 (6)	5.7
Chub Lake	Apr. 20/78	4.7 (1)	5.7
	Apr. 26/78	5.0 (3)	
Crosson Lake	Apr. 26/78	5.2 (5)	6.0
Fawn Lake	Apr. 10/78	6.3 (1)	5.8
	Apr. 26/78	4.7 (5)	

taken in each lake's littoral zone. The decrease in comparison with measurements made during late winter under ice cover ranges from 0.7 to 1.0 pH units. The sampling technique employed does not permit evaluation of the thickness of the acidic layer, although Scheider et al. (1979) previously reported a decline to a pH of 4.2 in the top 5 m of Fawn Lake in April 1975. Acidic surface layers reported in lakes in Sweden, Norway, and the Sudbury area of Ontario (Hultberg 1976; Henriksen and Wright 1977; Beamish 1974; Yan and Stokes 1978) had comparable pH depressions and varied in depth from 1 m to, in one case, the entire depth of the lake (Carlyle Lake, 12 m). The thickness of the layer will be a function of lake morphometry, meteorological factors affecting physical mixing, and relative magnitude of the runoff volume to the size of the lake (i.e. A_d/A_o = watershed area/lake area).

Consequences of Acidification

A large portion of south–central Ontario may potentially be affected by short-term snowmelt induced reduction of pH in streams and littoral zones of lakes. This is a result of two factors: (a) south–central Ontario has by mid-March of each year typically 1.0–1.5 m of snow cover with a pH of 4.0–4.3 (Scheider et al. 1979), and (b) as discussed by Kramer (1975), and Dillon et al. (1978a), many of the watersheds are underlain by Precambrian bedrock with only thin and scattered deposits of surficial tills of limited H^+ assimilation capacity. Furthermore, the spring melt may proceed quickly and run off over partially frozen soils with only partial neutralization taking place, so that the pH's of streams are reduced in even moderately well-buffered watersheds.

The effects of long-term acidification on the chemistry and biota of lakes have been well studied (Gjessing et al. 1976; Leivestad et al. 1976; Beamish and Harvey 1972; Dickson 1975; Almer et al. 1974; Dillon et al. 1978b). The impact of short-term depressions of pH, such as those occurring during snowmelt, are less well known. Consequently, changes in biota may occur be-

fore traditional sampling programs identify a permanent decline in pH in lakes. Although we did not attempt to evaluate the effect of exposure to acidic snowmelt on the biota, other studies have identified some likely ramifications. Börgström and Hendrey (1976) found that exposures of *Gammarus lacustris* to pH <5.5 in soft water for 45 h killed the majority of the test animals. Survival and emergence of many aquatic insects is reduced by short exposures to low pH waters (Bell 1971). While Bell's tests were conducted at 18.5°C, it is possible that the results may be extrapolated to lower water temperatures.

Littoral zones and tributaries of lakes in central Ontario are both spawning and nursery grounds for several fish species including lake trout (*Salvelinus namaycush*), brook trout (*S. fontinalis*), lake whitefish (*Coregonus clupeaformis*), walleye (*Stizostedion vitreum vitreum*), and others. These populations may be affected by the low pH conditions of spring runoff. The yolk-sac stage, which appears to be the most sensitive life period for salmonids (Snekvik 1969 in Hendrey and Wright 1976), often coincides with snowmelt (Scott and Crossman 1973). In a laboratory simulation of winter egg incubation at pH 8.0 and spring hatch, Trojnar (1977) found that the survival of brook trout fry decreased significantly when exposed to water at pH <5.0. In the extreme case large pH reductions during snowmelt may also result in major adult fish kills such as the one observed in the Tovdal River, Norway, during March 1975 (summarized in Leivestad et al. 1976). Unfortunately, some of the most sensitive fish species in central Ontario are highly prized sport fish such as the salmonids. The fact that we can only speculate on possible ecological effects of acidic snowmelt emphasizes the need for future studies to assess this impact.

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ALMER, B., W. DICKSON, E. EKSTRÖM, AND E. HÖRNSTRÖM, 1974. Effects of acidification on Swedish lakes. Ambio 3: 30–36.

1978. Sulfur pollution and the aquatic ecosystem, p. 273–311. *In* J. Nriagu [ed.] Sulfur in the Environment, Part II. Ecological impacts. J. Wiley and Sons, Inc., New York, N.Y.

BEAMISH, R. J. 1974. The loss of fish populations from unexploited remote lakes in Ontario, Canada as a consequence of atmospheric fallout of acid. Water Res. 8: 85–95.

BEAMISH, R. J., AND H. H. HARVEY. 1972. Acidification of the La Cloche Mountain lakes, Ontario and resulting fish mortalities. J. Fish. Res. Board Can. 29: 1131–1143.

BELL, H. L. 1971. Effects of low pH on the survival and emergence of aquatic insects. Water Res. 5: 313–319.

BÖRGSTRÖM, R., AND G. R. HENDREY. 1976. pH tolerance of the first larval stages of *Lepidurus arcticus* (Pallas) and adult *Gammarus lacustris* G. O. Sars. Sur Nedbors Virkning På Skog og Fisk IR 22/76: 37 p.

DICKSON, W. 1975. The acidification of Swedish lakes. Rep. Inst. Freshwater Res. Drottningholm 54: 8–20.

DILLON, P. J., D. S. JEFFRIES, W. SNYDER, R. REID, N. D. YAN, D. EVANS, J. MOSS, AND W. A. SCHEIDER. 1978a. Acid precipitation in south-central Ontario: recent observations. J. Fish. Res. Board Can. 35: 809–815.

DILLON, P. J., N. D. YAN, W. A. SCHEIDER, AND N. CONROY. 1978b. Acidic lakes in Ontario: characterization, extent, and responses to base and nutrient additions. Ergebnisse der Limnologie. Proceedings of a Conference on Lake Metabolism and Management. Uppsala, Sweden, Aug. 1977.

GJESSING, E. T., A. HENRIKSEN, M. JOHANNESSEN, AND R. F. WRIGHT. 1976. Effects of acid precipitation on freshwater chemistry, p. 64–85. *In* F. Braekke [ed.] Impact of acid precipitation on forest and freshwater ecosystems in Norway. Sur Nedbors Virkning På Skog og Fisk Res. Rep. 6/76.

GRÖTERUD, O. 1972. Zooplankton and fish in relation to acid melt water and anaerobic deep water in a lake. Vatten 28: 329–332.

HAAPLA, H., P. SEPPONEN, AND E. MESKUS. 1975. Effects of spring floods on water acidity in the Kiiminkijoki area, Finland. Oikos 26: 26–31.

HENDREY, G. R., AND R. F. WRIGHT. 1976. Acid precipitation in Norway: effects on aquatic fauna. J. Great Lakes Res. Suppl. 1, Proc. Symp. Atmospheric Contributions to the Chemistry of Lake Waters 2: 192–207.

HENRIKSEN, A., AND R. F. WRIGHT. 1977. Effects of acid precipitation on a small acid lake in southern Norway. Nord. Hydrol. 8: 1–10.

HULTBERG, H. 1976. Thermally stratified acid water in late winter — a key factor inducing self-accelerating processes which increase acidification. Water Air Soil Pollut. 7: 279–294.

JOHANNESSEN, M., T. DALE, E. T. GJESSING, A. HENRIKSEN, AND R. F. WRIGHT. 1977. Acid precipitation in Norway: the regional distribution of contaminants in snow and the chemical concentration processes during snowmelt. Proc. Isotopes and Impurities in Snow and Ice Symposium, Grenoble 1975. Int. Assoc. Hydrol. Sci. Publ. 118: 116–120.

JOHANNESSEN, M., AND A. HENRIKSEN. 1977. Chemistry of snowmelt water changes in concentration during melting. Sur Nedbors Virkning På Skog og Fisk. IR 11/77: 11 p.

KRAMER, J. R. 1975. Geochemical and lithological factors in acid precipitation. Proc. 1st Int. Symp. on acid precipitation and the forest ecosystem. U.S. For. Serv. Gen. Tech. Rep. NE 23: 611–618.

LEIVESTAD, H., G. HENREY, I. P. MUNIZ, AND E. SNEKVIK. 1976. Effects of acid precipitation on freshwater organisms, p. 87–111. *In* F. Braekke. [ed.] Impact of acid precipitation on forest and freshwater ecosystems in Norway. Sur Nedbors Virkning På Skog og Fisk Res. Rep. 6/76.

LIKENS, G. E., F. H. BORMANN, R. S. PIERCE, J. S. EATON, AND N. M. JOHNSON. 1977. Biogeochemistry of a forested ecosystem. Springer-Verlag, N.Y. 164 p.

MUNIZ, I. P., H. LEIVESTAD, E. T. GJESSING, E. JORANGER, AND D. SVALASTOG. 1975. Fiskedod i forbindelse med snøsmelting i Tovdalsvassdraget våren 1975. Sur Nedbors Virkning På Skog og Fisk IR 13/75: 60 p.

ODÉN, S., AND T. AHL. 1970. The acidification of Scandinavian waters. Ymer. Arsbok: 103–122.

ONTARIO MINISTRY OF THE ENVIRONMENT. 1975. Outlines of analytical methods. Tech. Rep.

PENTLAND, R. L. 1968. Runoff characteristics in the Great Lakes Basin. Proc. 11th Conf. Great Lakes Res. Int. Assoc. Great Lakes Res. 326–359.

SCHEIDER, W. A., D. S. JEFFRIES, AND P. J. DILLON. 1979. Effects of acidic precipitation on Precambrian freshwaters in southern Ontario. J. Great Lakes Res. (In press)

SCHINDLER, D. W., R. W. NEWBURY, K. G. BEATY, AND P. CAMPBELL. 1976. Natural water and chemical budgets for a small Precambrian lake basin in central Canada. J. Fish. Res. Board Can. 33: 2526–2543.

SCOTT, W. B., AND E. J. CROSSMAN. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184: 966 p.

TROJNAR, J. R. 1977. Egg hatchability and tolerance of brook trout (*Salvelinus fontinalis*) fry at low pH. J. Fish. Res. Board Can. 34: 574–579.

YAN, N. D., AND P. STOKES. 1978. Phytoplankton of an acidic lake, and its responses to experimental alterations of pH. Environ. Conser. 5: 93–100.

Population Dynamics of Spiny Dogfish (*Squalus acanthias*) in British Columbia Waters

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An age-structure model that incorporates recent information regarding growth and reproduction has been developed to investigate the dynamics of spiny dogfish (*Squalus acanthias*) populations in British Columbia waters. Mechanisms for density-dependent regulation of abundance were evaluated on the basis of theoretical effectiveness and ability to predict observed patterns in simulations of the historical fishery. Compensatory change in the rate of natural mortality appears to be the principal mechanism for density-dependent response. The instantaneous rate of natural mortality at natural equilibrium was estimated to be 0.094. MSY for British Columbia waters is ~9000–11 000 t/yr. The minimum size at entry to the fishery has little effect on MSY. Sustained-effort, sustained-yield, and periodic fisheries were modeled to determine optimum harvesting strategies. Periodic fisheries were found to vary widely in feasibility and appear to be impractical from a management perspective whereas a sustained-effort fishery would ensure maximum stability.

Key words: spiny dogfish, population dynamics, age-structure model, density-dependence, simulation model, commercial fishing strategies

WOOD, C. C., K. S. KETCHEN, AND R. J. BEAMISH. 1979. Population dynamics of spiny dogfish (*Squalus acanthias*) in British Columbia waters. J. Fish. Res. Board Can. 36: 647–656.

Nous avons élaboré un modèle de structure d'âge incorporant de récentes connaissances sur la croissance et la reproduction en vue d'étudier la dynamique des populations d'aiguillats (*Squalus acanthias*) dans les eaux de la Colombie-Britannique. Nous évaluons les mécanismes de contrôle de l'abondance liés à la densité sur la base de l'efficacité et de l'aptitude théorique à prédire, à l'aide de simulations de la pêcherie historique, ce qui se produit en réalité. Le principal mécanisme d'une réponse liée à la densité semble être un changement compensateur dans le taux de mortalité naturelle. On estime à 0,094 le taux instantané de mortalité naturelle à l'équilibre naturel. Le RMS dans les eaux de la Colombie-Britannique est de l'ordre de 9 000–11 000 t/an. La taille minimale au moment de l'entrée dans la phase exploitable a peu d'effet sur le RMS. Nous construisons des modèles de pêcheries à effort soutenu, à rendement soutenu et de pêcheries périodiques afin d'arrêter des stratégies de récolte optimale. On constate que les pêcheries périodiques accusent de fortes variations quant à leur praticabilité et semblent peu pratiques du point de vue de leur gestion. Une pêcherie à effort soutenu, d'autre part, assurerait une stabilité maximale.

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THE spiny dogfish (*Squalus acanthias*) has not been exploited heavily in British Columbia waters since the intensive liver fishery of the 1940s. Because of renewed interest, we have reviewed the biology of dogfish and the history of its fishery in the northeastern Pacific to assess the overall potential of this resource. Experience with other shark fisheries indicates that most often initial exploitation is followed by a rapid decline in catch rates and sometimes a complete collapse (Holden 1974). Although many aspects of dogfish biology in the northeastern Pacific have been investigated (Bonham et al. 1949; Ketchen 1972, 1975; Jones and Geen 1977a–d), there has been no comprehensive analysis to determine the potential for exploitation.

The dogfish, like other sharks, is an extremely long-lived, slow-growing species whose reproductive capacity is limited by late maturity, long gestation period, and low fecundity. Consequently, many accepted models for stock assessment are not applicable. In particular, stock production models that assume that the rate of natural increase responds immediately to changes in population density and that the rate of natural increase at a given density is independent of the age composition of the stock are inappropriate since these assumptions are obviously not satisfied (Holden 1977). An alternative approach explored in this paper was to develop a deterministic age-structure model that incorporates all avail-

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able information regarding growth, maturity, and fecundity of dogfish in British Columbia waters.

Age-Structure Model

Age-structure models are a class of dynamic population models that incorporates age-specific survival and reproductive parameters and may include some mechanism for compensation providing surplus production at low population densities. This type of model is ideally suited for simulations of populations characterized by age-specific reproductive capacity and marked fluctuations in year-class strength. They have been used widely in investigations of mammal populations (e.g. Caughley 1977) but also as management models for some fisheries (e.g. Southward 1968; Walters 1969). For the same reasons, age-structure analysis has also been useful for investigating the dynamics of dogfish populations.

Equilibrium Population

Reproductive potential — Mean length-at-age data from von Bertalanffy growth equations for the Strait of Georgia (Ketchen 1975) were used to determine age-specific reproductive potentials from percent maturity – length and fecundity – length relationships described by Ketchen (1972). However, since eggs begin development 4 yr before birth (Ford 1921), the regression of fecundity on length was transformed to predict the average number of developing eggs rather than the average litter size at birth for any given length (equation 1). This was necessary to simulate the time lag between increased litter sizes and density-dependent increases in growth rate discussed in a later section.

$$f_{i,x} = -9.96 + 0.176\, l_{i,x} \tag{1}$$

where $f_{i,x} \geq 0$ and represents the number of eggs developing in a female dogfish of length, $l_{i,x}$ (in cm) at age i in year x. Thus, the average litter size of a given age-class i in year x is given by $f_{i-1,\ x-1}$. Because the gestation period (after fertilization) is nearly 2 yr (Ford 1921), only those females possessing mature eggs 2 yr previous are able to produce offspring; this also implies that only about half the number of mature females in each age-class will give birth each year. For an equal sex ratio (Ford 1921) (requiring further division by 2), the age-specific reproductive potential ($R_{i,x}$) is given in equation (2).

$$R_{i,x} = \tfrac{1}{4}\, m_{i-2,\ x-2}\, f_{i-1,\ x-1} \tag{2}$$

where $m_{i-2,x-2}$ represents the percentage of mature females of age i-2 in year x-2 and hence is the percentage capable of producing offspring in year x.

Natural morality — No quantitative information is available regarding age-specific rates of natural mortality for dogfish. However, it has been assumed that natural mortality is equal for all age-groups since there is some evidence that this is true for other long-lived species (Beverton and Holt 1960).

At equilibrium in the absence of fishing mortality (here denoted by ^), the rate of births will equal the rate of natural mortality. Assuming that the reproductive parameters of the present, relatively unexploited population are similar to those for the equilibrium population, the instantaneous rate of natural mortality at equilibrium ($\hat{M}$) was determined to be 0.094 by solving simultaneous equations (3) and (4) by iteration,

$$\hat{n}_t = \hat{n}_o\, e^{-\hat{M}t} \tag{3}$$

$$\hat{n}_o = \sum_{t=1}^{60} \hat{n}_t\, \hat{R}_t \tag{4}$$

where $\hat{n}_o$ is an arbitrary number of fish of age o, $\hat{n}_t$, the number of fish of age i and $\hat{R}_t$, the reproductive potential of age-class i in the equilibrium population ($\hat{N}$).

Nonequilibrium Population

Density-dependence — In any population, surplus production must occur at some density of reproductive stock or at some time in its history; otherwise the stock could not endure changes in abundance associated with life in an unpredictable environment and in particular, exploitation by a fishery. For most fish populations, it is generally accepted that surplus production occurs through enhanced survival of eggs and larvae under favorable conditions at the expense of reproductive waste during unfavorable periods. However, it is unlikely that environmentally determined mortality is a major factor in controlling dogfish abundance (Holden 1973). Dogfish embryos develop within the uterus, where they are protected from environmental influences; at birth, they are large (23–30 cm) and presumably better able to withstand or avoid adverse environments than the planktonic larvae of other teleost fishes. Moreover, the reproductive capability of a dogfish stock is severely limited by the number of breeding females and there is little margin for high or variable environmental mortality given the already low rate of natural mortality estimated for equilibrium conditions ($\hat{M} = 0.094$). Hence, it is presumed that some form of density-dependent response must exist.

Holden (1973) has speculated about possible mechanisms for density-dependence in dogfish populations. These can be categorized as (1) compensatory decreases in natural mortality perhaps resulting from decreased predation, cannibalism, competition, or disease, (2) compensatory increases in fecundity when food is more readily available or through decreased uterine mortality, and (3) compensatory increases in growth rate when food is more abundant resulting in earlier maturity and greater fecundity for each age-class (assuming that fecundity and maturity are related to size rather than age). Whereas compensatory mortality may act upon many age-classes simultaneously, compensatory changes in reproductive potential act initially on individual year-class strengths and only subsequently on the overall age structure as these cohorts grow to maturity. The size of the body cavity places an obvious constraint on compensatory increases in fecundity. Furthermore, because eggs develop at least 4 yr before parturition, there would be a considerable lag in the response time of both compensatory fecundity and compensatory growth mechanisms. Another time lag is introduced by the cumulative effect of changes in growth rate; once individuals of an age-class have grown quickly to a large size, they will remain at least as large for the duration of their life span.

Since few data are available to support or deny the existence of any of these mechanisms, we have investigated each in turn using the following linear functions (equations 5–8):

i) compensatory mortality:

$$\nu_{i,x} = \left(\alpha + \beta \frac{N_x}{\hat{N}}\right)\hat{\nu}_i \tag{5}$$

hence in the absence of fishing,

$$M_{i,x} = -\log_e\left[1 - \left(\alpha + \beta\frac{N_x}{\hat{N}}\right)\left(1 - e^{-\hat{M}_i}\right)\right] \tag{6}$$

where $\nu_{i,x}$ and $\hat{\nu}_i$ are the annual rates of natural mortality and $M_{i,x}$ and $\hat{M}_i$ are the instantaneous rates of natural mortality at age i for the population in year x and the equilibrium population, respectively. N_x and $\hat{N}$ represent the abundance during year x and at equilibrium.

ii) compensatory fecundity:

$$R_{i,x} = \left(\alpha - \beta\frac{N_x}{\hat{N}}\right)\hat{R}_i \tag{7}$$

and iii) compensatory growth:

$$\Delta l_{i,x} = \left(\alpha - \beta\frac{N_x}{\hat{N}}\right)\Delta\hat{l}_i \tag{8}$$

where $R_{i,x}$ and $\hat{R}_i$ are the age-specific reproductive potentials and $\Delta l_{i,x}$ and $\Delta\hat{l}_i$, the average annual length increments of age-class i in year x and at equilibrium, respectively. The constants α and β determine the degree of compensation each mechanism can provide and were selected from a range of plausible values to determine the relative effectiveness of each mechanism.

Computation with fishing mortality — The computation of future dogfish populations follows the fecundity–survival matrix model (Leslie 1945). Age-specific survival rates ($S_{i,x}$), incorporating fishing mortality ($F_{i,x}$) and natural mortality ($M_{i,x}$), determine the age structure of the population at the end of year x–beginning of year $x + 1$ (equations 9 and 10). The magnitude of the 0 age-class for year $x + 1$ is dependent on the abundance of the reproductive age-classes and their reproductive potential at the end of year x (i.e. $x + 1$) since parturition occurs in late fall (equation 11).

$$n_{i+1,\,x+1} = n_{i,x}S_{i,x} \tag{9}$$

$$\text{where } S_{i,x} = e^{-(M_{i,x} + F_{i,x})} \tag{10}$$

$$n_{0,\,x+1} = \sum_{i=0}^{59} n_{i+1,\,x+1}\,R_{i+1,\,x+1} \tag{11}$$

Simulation of the Fishery

Recruitment to fishable stock — Historically, dogfish <70 cm (16 yr) are not represented in catches by sunken gillnet and only to a small extent in catches by otter trawl (Barraclough 1948a; Jones and Geen 1977c). There is also evidence that juvenile dogfish are found predominately in the midwater region (Beamish and Smith 1976) which explains how they escape capture by bottom trawl. Hence, in this analysis they have been regarded as not vulnerable to a fishery. Recruitment to the fishery has been considered to be by platoon such that dogfish between 73–80 cm (16–20 yr) experience only half the fishing mortality sustained by those >80 cm, based roughly on size composition of catches during past fisheries. Although large female dogfish were most valuable during the early years of the liver fishery because they possessed the largest livers, vitamin potency tests determined prices in the latter part of the fishery; since it has been determined that for equal-sized dogfish, livers from males are more potent than those from females (Bonham et al. 1949), it is likely that both sexes experienced comparable fishing rates over the course of the fishery. Furthermore, male and female dogfish grow at similar rates to at least 80 cm and appear to be exploited equally in the present food-fishery.

Exploitation rates from catch data — Catch and effort data from the liver fishery of the 1940s have been used to estimate the size of the dogfish stock(s) at the outset of the fishery. By relating fishing success to cumulative fishing effort (DeLury's method 1947) and fishing success to cumulative catch (method of Leslie and Davis 1939), the dogfish stock(s) from northern British Columbia to Oregon including the Strait of Georgia and Puget Sound was estimated to be ~300 000–500 000 t of marketable-sized fish (Ketchen 1969). For the purposes of the present analysis, it has been assumed that roughly half of this biomass (200 000 t) inhabited British Columbia waters. Since catches off British Columbia during the 30 yr before the liver fishery averaged <800 t/yr, the population was considered to be at natural equilibrium before 1937. Thus, the model population can be related to the British Columbia stock by a scaling factor equal to the ratio of the estimated virgin biomass of marketable stock off B.C. (200 000 t) to the marketable biomass of the equilibrium model population ($\sum_{i=16}^{60} \hat{n}_i\hat{w}_i$ where $\hat{n}_i$ and $\hat{w}_i$ represent the abundance and average weight of fish at age i in the equilibrium population). In this manner British Columbia catch data were expressed as a simulation catch (Y_x^*) in terms of the model population. Exploitation rates for fully recruited age-classes ($\mu_{21-60,x}$) and corresponding instantaneous rates of fishing mortality ($F_{i,x}$) were estimated in accordance with the foregoing assumptions about recruitment (equations 12–14).

$$\mu_{21-60,x} = \frac{Y_x^*}{\frac{1}{2}\sum_{i=16}^{20} n_i w_i + \sum_{i=21}^{60} n_i w_i} \tag{12}$$

$$F_{21-60,x} = -\log_e[1 - \mu_{21-60,x}] \tag{13}$$

$$F_{16-20,x} = \tfrac{1}{2}F_{21-60,x} \tag{14}$$

Equation (13) is strictly true only in the absence of natural mortality but is a good approximation where growth compensates for natural mortality to some extent during the fishing season. In fact, yield (Y_x) calculated from these estimated rates of fishing mortality using equations (15)–(17) never deviated from the simulation catch (Y_x^*) by >5%.

$$Y_x = \sum_{i=0}^{60} F_{i,x}\,\bar{n}_{i,x}\,\bar{w}_{i,x} \tag{15}$$

$$\bar{n}_{i,x} = \frac{n_{i,x}\,A_{i,x}}{Z_{i,x}} \tag{16}$$

$$\bar{w}_{i,x} = \frac{w_{i,x}\,(e^{G_{i,x}} - 1)}{G_{i,x}} \tag{17}$$

where $n_{i,x}$ and $w_{i,x}$ represent the number of fish and their corresponding average weight at age i at the beginning of year x and $\bar{n}_{i,x}$ and $\bar{w}_{i,x}$ the average number of fish and their corresponding average weight at age i during year x. $A_{i,x}$ and $Z_{i,x}$ are the annual rate and instantaneous rate of total mortality for age i in year x, respectively, and $G_{i,x}$ is the instantaneous rate of growth (in weight) of age i in year x. Weight at age was calculated separately for each sex by relating mean length-at-age data to weight–length relationships reported by Jones and Geen (1977b) and averaged to obtain the mean weight of individuals in each age-class ($w_{i,x}$). The time index (x) becomes necessary when growth is assumed to be density-dependent. Estimates of yield, biomass, and abundance were related to British Columbia waters by means of the scaling factor.

Results and Management Projections

Natural Mortality and Initial Estimates of MSY

Solving equations (3) and (4) provided an estimate of the instantaneous rate of natural mortality ($\hat{M}$) of 0.094 for the equilibrium population. For the majority of fish stocks, the rate of fishing mortality resulting in maximum sustainable yield (MSY) is very nearly equal to the rate of natural mortality in the unexploited population. From this observation Gulland (1970) has proposed an approximate formula for estimating maximum sustainable yield:

$$\text{MSY} \approx X M B_0 \tag{18}$$

where B_0 is the virgin exploitable biomass, M is the instantaneous rate of natural mortality, and X is the maximum fraction that can be captured from the biological production of the stock and equal to 0.5 in the Schaefer production model. If this rationale is applied, noting that some of the assumptions of the Schaefer model are not satisfied and that the inferred relationship between optimum fishing mortality and natural mortality has never been demonstrated for an elasmobranch fishery, MSY for dogfish in British Columbia waters is estimated to be ~8000–10 000 t/yr.

Density-Dependent Response

In this section, an attempt has been made to ascertain what mechanism of density-dependence operates in dogfish populations of the northeastern Pacific.

Theoretical effectiveness — The first step in this procedure was to identify which of the mechanisms under consideration was most effectual, i.e. could provide the greatest compensation over a range of plausible values of α and β in equations (5)–(8). Compensatory fecundity alone cannot provide sufficient response since an increase of 500% (clearly absurd) would be required to sustain a fishery of only 5000 t/yr off British Columbia (Fig. 1). Moreover, due to the time lag during egg development, the model population becomes unstable at low fishing rates.

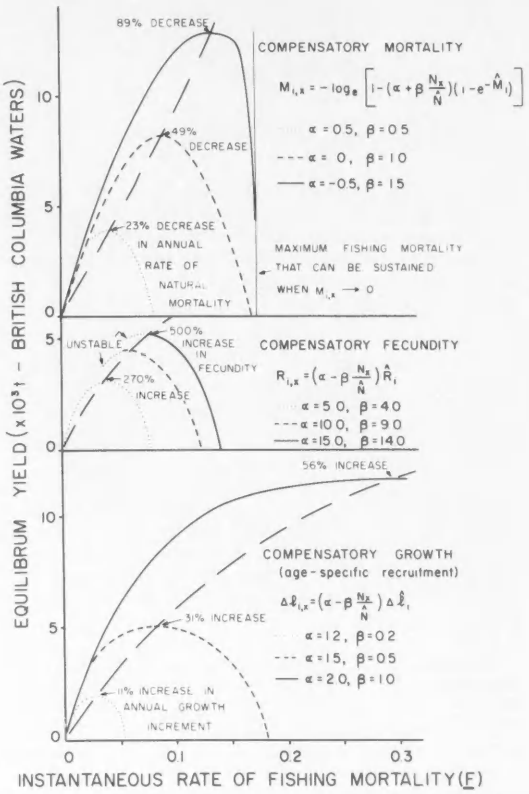
89% DECREASE
COMPENSATORY MORTALITY
$M_{i,x} = -\log_e\left[1-(\alpha+\beta\frac{N_x}{\hat{N}})(1-e^{-\hat{M}_i})\right]$
α = 0.5, β = 0.5
--- α = 0, β = 1.0
— α = -0.5, β = 1.5
49% DECREASE
23% DECREASE IN ANNUAL RATE OF NATURAL MORTALITY
MAXIMUM FISHING MORTALITY THAT CAN BE SUSTAINED WHEN $M_{i,x} \to 0$
UNSTABLE
500% INCREASE IN FECUNDITY
270% INCREASE
COMPENSATORY FECUNDITY
$R_{i,x} = (\alpha-\beta\frac{N_x}{\hat{N}})\hat{R}_i$
α = 5.0, β = 4.0
--- α = 10.0, β = 9.0
— α = 15.0, β = 14.0
56% INCREASE
COMPENSATORY GROWTH
(age-specific recruitment)
$\Delta\hat{\ell}_{i,x} = (\alpha-\beta\frac{N_x}{\hat{N}})\Delta\hat{\ell}_i$
31% INCREASE
α = 1.2, β = 0.2
--- α = 1.5, β = 0.5
— α = 2.0, β = 1.0
11% INCREASE IN ANNUAL GROWTH INCREMENT
EQUILIBRIUM YIELD (x 10³ t – BRITISH COLUMBIA WATERS)
INSTANTANEOUS RATE OF FISHING MORTALITY (F)
0
5
10
0.1
0.2
0.3

FIG. 1. Relationship between equilibrium yield, fishing mortality, and degree of compensation from three density-dependent mechanisms. Dashed lines represent loci for MSY for other values of α and β in compensatory functions.

A compensatory growth mechanism can provide greater sustainable yields by increasing both fecundity and the percentage mature at age without changing fecundity–length or maturity–length relationships (Fig. 1). Whereas an 11% increase in annual growth increments at the new equilibrium would sustain removals of only 2000 t/yr, a 56% increase would sustain a fishery of nearly 12 000 t/yr. The yield curves for compensatory growth in Fig. 1 assume that recruitment to the fishery is age-specific regardless of the fact that with a faster growth rate, fish reach the "catchable" size range at an earlier age. Further analysis showed that MSY for length-specific recruitment is similar to that for age-specific recruitment except that the optimum fishing mortality to maintain MSY is much lower. Thus, whether recruitment to the fishery depends on age or size, an increase in growth of 40–50% would be required to provide the expected MSY of 8000–10 000 t/yr. At this accelerated rate, the weight of a 60-yr-old fish (male and female weights averaged) would increase

from 4.5 to 10–11 kg. However, due to fishing mortality, the oldest surviving fish would be only 35 yr (weighing 7 kg compared to 3.1 kg at present) with age-specific recruitment or 45 yr (weighing 8.5 kg compared to 3.8 kg at present) with length-specific recruitment. To say the least, it is questionable whether an increase in growth of this magnitude is possible.

On the other hand, an MSY of 8000–10 000 t/yr is possible through a 40–50% reduction in the annual rate of natural mortality for all age-classes. Because the rate of natural mortality is already low in the absence of fishing ($\hat{M} = 0.094$), this reduction is small in absolute terms — 5% rather than 9% of the stock would die each year. Another consequence of the compensatory mortality model is that the maximum age attained under optimum rates of exploitation would decrease only slightly, remaining close to 60 yr opposed to 35 or 45 yr with compensatory growth. If, however, the mortality rate is assumed to be density-dependent for juvenile dogfish (0–15 yr) alone, MSY is reduced drastically; a 50% reduction in the annual rate of natural mortality would sustain removals of only 3000 t/yr and a reduction of at least 80% would be required to provide an MSY of 6000 t/yr.

Simulation results — In the next step of the analysis, we have attempted to deduce which mechanism has the greatest regulatory influence on dogfish abundance by comparing predictions from simulations of the historical fishery with recent observations. Only the latter two mechanisms have been considered since it has already been demonstrated that compensatory changes in fecundity alone can only be of minor significance. Three compensatory functions capable of providing a range of surplus production — an MSY of 9000 t/yr as estimated for British Columbia with an upper limit of 12 000 t/yr and a lower limit of 5000 t/yr — were used for each mechanism in the simulations. Recruitment to the fishery was considered to be length-specific based on available size composition data from historical catches. In Fig. 2, it appears that the decline in abundance of marketable stock during the fishery is relatively unaffected by the degree of compensation of either mechanism. Marketable stock was reduced to ~55% of its unexploited abundance (44% of the unexploited biomass, B_o) by 1949 in the compensatory mortality model but to only 68% of its virgin abundance (74% of B_o) in the compensatory growth model. A striking feature of the compensatory mortality simulation is the rapid recovery of marketable stock which is predicted to have reached a peak of 82–128% of its unexploited biomass by 1960 depending on the degree of compensation. No such recovery is predicted by the compensatory growth model since the recovery is a consequence of enhanced survival of juvenile dogfish that reach marketable size shortly after the simulated liver fishery has ended. Catch statistics from the sunken gillnet fishery in Hecate Strait (Barraclough 1948a, b) which represented nearly a third of the total removals from British Columbia waters, suggest that by 1948 the biomass of marketable dogfish had been reduced to about one quarter of its primitive size (Ketchen 1969). These statistics indicate that the stock(s) could not sustain a fishery that averaged 15 000 t/yr over a period of 13 yr. Yet as early as 1959, the Canadian Department of Fisheries, responding to a growing number of complaints about the menace of dogfish to other fishing operations, introduced a subsidy program to reduce the dogfish population. This action and other anecdotal reports attest to a surprising increase in dogfish abundance during the 1950s which lends some credibility to the compensatory mortality model. Alternatively, it is possible that the apparent recovery resulted from immigration from less exploited populations.

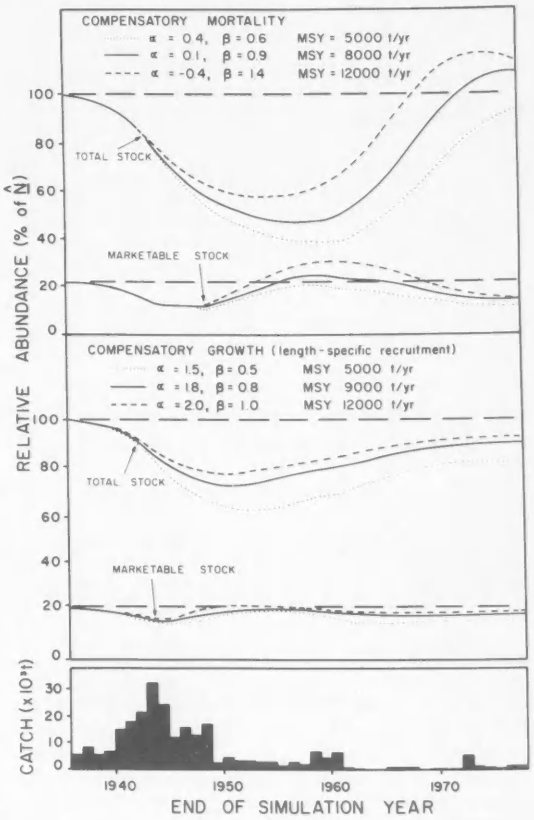
COMPENSATORY MORTALITY
α = 0.4, β = 0.6 MSY = 5000 t/yr
α = 0.1, β = 0.9 MSY = 8000 t/yr
α = -0.4, β = 1.4 MSY = 12000 t/yr
TOTAL STOCK
MARKETABLE STOCK
RELATIVE ABUNDANCE (% of $\hat{N}$)
COMPENSATORY GROWTH (length-specific recruitment)
α = 1.5, β = 0.5 MSY 5000 t/yr
α = 1.8, β = 0.8 MSY 9000 t/yr
α = 2.0, β = 1.0 MSY 12000 t/yr
TOTAL STOCK
MARKETABLE STOCK
CATCH (x10⁴t)
1940
1950
1960
1970
END OF SIMULATION YEAR

FIG. 2. Trends in abundance predicted by simulations of the historical fishery based on compensatory mortality and compensatory growth models.

While both models predict that the stock is presently approaching its historic level, there are marked differences in age composition projected for 1978 (Fig. 3). Empirical age data collected from over 4000 dogfish

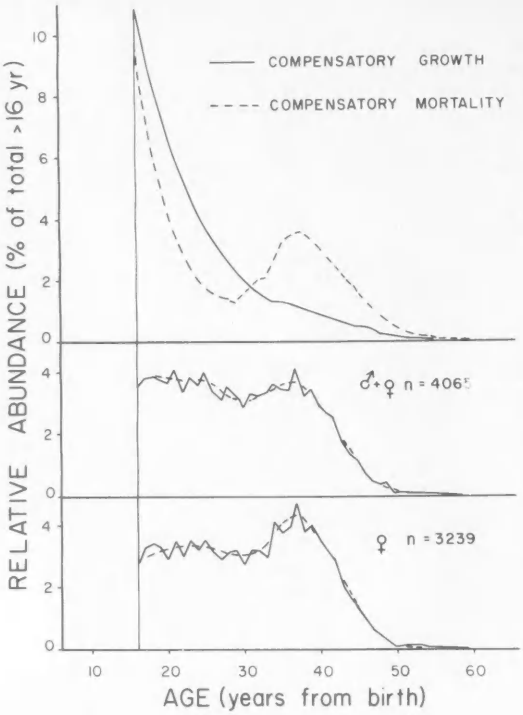

FIG. 3. Comparison of age structures predicted for 1978 in simulations based on compensatory mortality and compensatory growth models with empirical data collected from Hecate Strait during 1978 (Beamish et al. unpublished data).

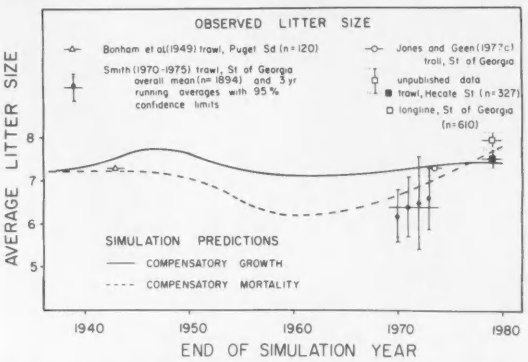
OBSERVED LITTER SIZE
Bonham et al(1949) trawl, Puget Sd (n=120)
Smith (1970-1975) trawl, St of Georgia overall mean (n=1894) and 3 yr running averages with 95% confidence limits
Jones and Geen (1977c) trawl, St of Georgia
unpublished data
trawl, Hecate St (n=327)
longline, St of Georgia (n=610)
SIMULATION PREDICTIONS
COMPENSATORY GROWTH
COMPENSATORY MORTALITY
AVERAGE LITTER SIZE
END OF SIMULATION YEAR

FIG. 4. Comparison of trends in average litter size predicted in simulations based on compensatory mortality and compensatory growth models with empirical data from northeastern Pacific during and after liver fishery. (Data reported by Smith 1970–75 include observations by Smith and Kristiansen 1970a, b.)

in Hecate Strait during 1978 show a distinct mode at 35–40 yr (Fig. 3) (Beamish et al. unpublished data). An identical mode is apparent in the age structure predicted for 1978 by the compensatory mortality model but is notably absent in that predicted by the compensatory growth model.

A comparison of predicted and observed average litter sizes furnishes additional evidence. Average litter size of the stock is dependent upon both the average fecundity of each age-class and upon their relative contribution to total reproduction as determined by the age composition of the population. The selective removal of older fish during a fishery tends to decrease the average litter size of the stock whereas an increase in growth rate tends to augment the average litter size. Consequently, there are noticeable differences in trends in average litter size predicted by each model. These are compared with observations made at various times since the beginning of the liver fishery in Fig. 4. Unfortunately no data were collected between 1950 and 1970 when the predicted trends differ most. However, the available data do contribute additional, though admittedly tenuous, support for a compensatory mortality mechanism.

To conclude, simulations with a mechanism for compensatory mortality successfully predicted the anomalous age composition of recent samples from Hecate Strait, trends in estimates of average litter size, and the surprising increase in dogfish abundance shortly after the liver fishery had collapsed, whereas simulations with a mechanism for compensatory growth did not. Moreover, it has been demonstrated that the compensatory mortality mechanism is capable of providing the greatest degree of density-dependent response with the least alteration of population parameters. Collectively, these results provide substantial evidence that compensatory change in the rate of natural mortality is the principal mechanism of density-dependence in dogfish populations of the northeastern Pacific.

MANAGEMENT PROJECTIONS

With knowledge of the mechanism for density-dependent response, the age-structure model can be used to make qualitative projections with direct implications for management of a new fishery.

Optimum size — Computations incorporating the compensatory mortality mechanism for dogfish off British Columbia indicate that MSY is relatively unaffected by minimum length of entry to the fishery at the corresponding optimum rate of fishing mortality (Fig. 5). However, the optimum rate of fishing mortality bears a curvilinear relationship to minimum size. At present, processing requirements limit the acceptable size of landed dogfish to those >81 cm (32 in.). Our results suggest that there are no biological grounds for enforcing a minimum size limit.

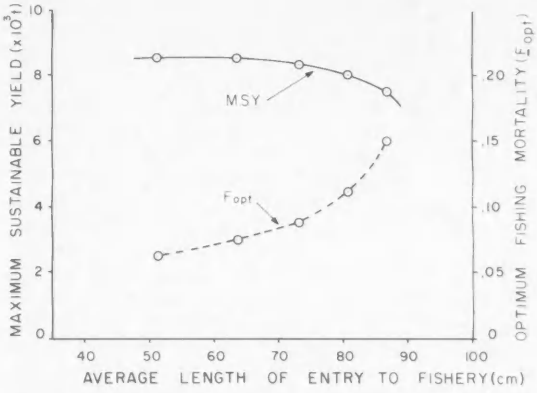

FIG. 5. Relationship between maximum sustainable yield (MSY), optimum rate of fishing mortality (F_{opt}), and length of entry to the fishery (average of male and female lengths) predicted by compensatory mortality model (with $\alpha = 0$, $\beta = 1.0$).

Refined estimates of MSY — Regardless of the degree of compensation in the compensatory mortality functions, MSY is attained when the marketable biomass has been reduced to 57% of its unharvested level. With reference to equation (18):

$$X = \frac{F_{\text{opt}}}{M} \cdot \frac{\bar{B}_{\text{opt}}}{B_o} \tag{19}$$

where M is the instantaneous rate of natural mortality of the unexploited populations, $\bar{B}_{\text{opt}}$ and F_{opt} are the marketable biomass and instantaneous rate of fishing mortality at MSY and B_o is the biomass of the unexploited marketable stock (Troadec 1978). Thus, with $\hat{M} = 0.09$ and $\bar{B}_{\text{opt}}/B_o = 0.57$, $X = 6.33\,F_{\text{opt}}$. Assuming $F_{\text{opt}} \approx \hat{M}$ (somewhere between 0.08 and 0.10) a refined estimate of MSY from equation (18) is 9000–11 000 t/yr.

Sustained vs. periodic harvesting strategies — Consequences of a sustained fishery averaging 8000–9000 t/yr are presented in Fig. 6 for three situations: (i) optimum exploitation ($F_{\text{opt}} = 0.09$, MSY = 8200 t/yr), (ii) underexploitation ($F_{\text{opt}} = 0.13$, MSY = 12 000 t/yr), and (iii) overexploitation ($F_{\text{opt}} = 0.05$, MSY = 5000 t/yr). In Fig. 6a, fishing effort is constant ($F = 0.09$), hence the predicted yield is equivalent to predicted catch-per-unit-effort, whereas in Fig. 6b an annual catch quota of 8200 t/yr is enforced as a more practical management alternative. It is apparent that the population is greatly affected by small changes in fishing mortality and that as a consequence of the time lag between reproduction and recruitment to the fishery, these perturbations perpetuate cycles of abundance. Therefore, greater stability can be ensured by regulating effort rather than catch, particularly if the estimate of MSY is too high.

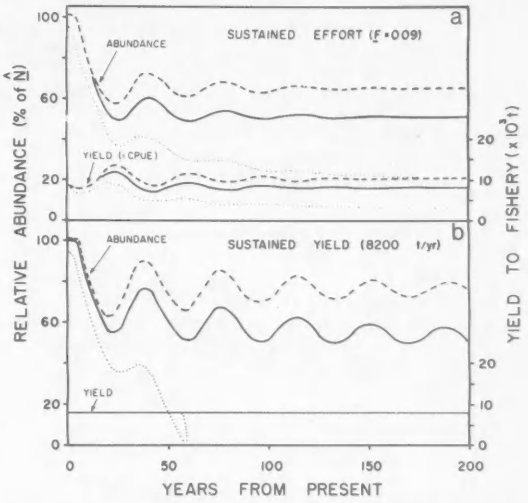

FIG. 6. Projected trends in abundance and yield for (a) a sustained-effort fishery ($F = 0.09$) where $F_{opt} = 0.09$, $F_{opt} = 0.13$, and $F_{opt} = 0.05$; (b) a sustained-yield fishery ($Y = 8200$ t/yr) where MSY = 8200 t/yr, MSY = 12 000 t/yr, and MSY = 5000 t/yr.

At present prices, the success of the dogfish fishery depends on a high catch-per-unit-effort. In view of the limited yield expected from a sustained fishery and because dogfish are still regarded as a nuisance to other fisheries, it appeared on first consideration that a more desirable strategy might be to fish intensively for short periods. Such a "periodic fishery" was expected to reduce the stock to a level of abundance at which dogfish would no longer be a menace and provide high catch rates for the duration of the fishery. Several periodic fishing strategies have been investigated by manipulating fishing effort, duration of the fishery, length of time between fisheries, and timing of the fishery with respect to stock abundance. The most successful of these trials is presented in Fig. 7 and demonstrates that it is theoretically possible to obtain high catch-per-unit-effort for several years at a time while maintaining the population at a reduced level of abundance during the intervening period. However, other trials were far less effective and some promoted great instability. This, in itself, implies that proper management of a periodic fishery would be extraordinarily difficult and the consequences of mismanagement, serious. Consequently, we recommend cautious development of a sustained-effort fishery.

Discussion

Although the analysis presented in this paper depends on a number of assumptions that are probably oversimplified, it does consolidate recent information about growth and reproduction of dogfish in a manner

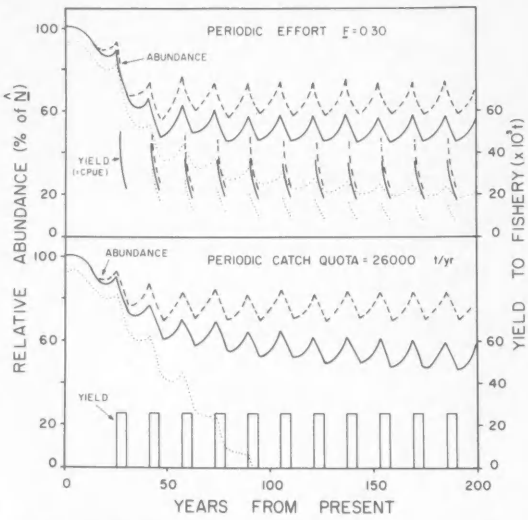

FIG. 7. Projected trends in abundance for a periodic fishery of 5 yr duration beginning every 16 yr with (a) constant effort during fishery ($F = 0.30$) and (b) a catch quota of 26 000 t/yr during fishery where MSY = 8200 t/yr, MSY = 12 000 t/yr, and MSY = 5000 t/yr.

that provides a greater understanding of the unique population dynamics of this species. The estimate of natural mortality for the equilibrium population, $M = 0.094$, is similar to estimates obtained from tag recovery data for the exploited Scottish–Norwegian stock which range from 0.16 (Aasen 1964) to 0.103 (Holden 1968). Unfortunately, no estimates of mortality from tagging data have been published for the northeastern Pacific. Since dogfish in the Pacific mature later and are longer-lived than Atlantic races (Ketchen 1972, 1975), it follows that they should experience a lower rate of natural mortality. This reasoning adumbrates the compensatory decrease in natural mortality expected under conditions of exploitation. Jones and Geen (1977c) have also estimated survivorship by calculating the number of female embryos required to replace one female at the modal length of mature fish. They concluded that in the absence of fishing mortality 36% of all females born die before reaching 35 yr of age which amounts to an annual mortality of only 1% or an instantaneous rate of 0.013. However, there are several problems with this analysis that may severely bias their estimate of natural mortality. They use empirical length-frequency data to construct a survivorship curve in order to estimate the numbers of age 0 fish produced. However, the length-frequency distribution is extrapolated over the range from 80 to 93 cm (Jones and Geen 1977c, fig. 7) and consequently the analysis loses its empirical basis. More importantly, their analysis includes a conversion from length to age, although this is not stated explicitly. There is no indication how such a conversion was made but it appears from the values in their life table that mean length-at-age data were used to determine the number of mature females at each age by reading directly off the length-frequency distribution. In other words, it appears that Jones and Geen have confused the length distribution with the corresponding age distribution in which case the resulting calculations are erroneous. By further assuming that the number of males and females are equal at their respective modal lengths, Jones and Geen also conclude that male dogfish experience a higher rate of natural mortality. However, their own empirical data (Jones and Geen 1977c, fig. 7, 8) do not indicate that equal numbers of males and females are found at their respective modal lengths and because of the different growth rates of mature males and females, there appears to be no basis for such an assumption.

Our analysis clearly suggests that compensatory change in the rate of natural mortality is a principal factor in the stock–recruitment relationship of dogfish in the northeastern Pacific. However, Holden (1973) appears to favor a mechanism for compensatory reproduction for the Scottish–Norwegian stock and argues that density-dependent changes in litter size would provide a greater degree of response than changes in the age at maturity. But his calculations do not consider how fishing mortality reduces the proportion of old fish in the population and their relative contribution to the total reproduction. Therefore increased fecundity has less and less effect on recruitment and earlier maturity, a greater effect, as fishing mortality increases. Holden cites the smaller average litter size of unexploited dogfish stocks off Newfoundland (4.2 per female) compared to that of the heavily exploited Scottish–Norwegian stock (5.78 per female) as evidence of increased fecundity in response to exploitation. But, in general, there appear to be great differences in reproduction and growth among geographically distinct populations (Ketchen 1972). Furthermore, there is no evidence that the relationship between average litter size and length has changed for dogfish in the northeastern Pacific over the past 30 yr following the collapse of the intensive liver fishery. During the fishery, the average number of uterine eggs and embryos for 90-, 100-, and 110-cm fish was 4.0, 6.4, and 8.8, respectively (Bonham et al. 1949), whereas the corresponding number of uterine embryos during the late 1960s and early 1970s (presumably a period of higher abundance) was reported to be 4.8, 6.8, and 8.8 (Ketchen 1972). Certainly, fecundity with respect to length has *not decreased* with increasing density in contrast to what would be expected if a compensatory mechanism for fecundity did exist as Holden (1973) suggests.

A surprising conclusion from the age-structure analysis is that MSY is relatively unaffected by minimum size limits. This is attributed to the species' slow rate of growth, low rate of natural mortality, and in particular to the direct nature of the stock–recruitment rela-

tionship. Holden (1968) has calculated the maximum permissible rate of total mortality and the corresponding yield-per-recruit for the Scottish–Norwegian stock over a range of minimum size limits such that reproduction is not diminished. His calculations indicate that yield-per-recuit increases in proportion to minimum length of entry up to ~95 cm. The discrepancy between our conclusions may be due, in part, to the differences in growth rate and size at maturity between the Scottish–Norwegian and the northeastern Pacific stocks, and also to our assumptions about density-dependence. Holden invokes the conservative assumption that the Scottish–Norwegian stock has reached its maximum capacity for recruitment under the prevailing rate of exploitation, whereas the density-dependent function of this analysis does not include such a threshold. Nevertheless, our results do concur with regard to the curvilinear relationship between optimum rate of fishing mortality and minimum size at entry.

It has been assumed in this study that linear density-dependent functions are sufficiently realistic to investigate the general properties of proposed mechanisms for density-dependence and to discriminate between probable and improbable mechanisms. Of course, a greater knowledge of the nature of mortality within dogfish populations may clarify these density-dependent relationships and permit formulation of more realistic curvilinear functions and age-specific mortality rates. The age-structure approach could also be extended to include patterns of distribution and migration as these become known. Fortunately, the dogfish stock(s) off British Columbia appear to have returned to a comparatively natural state and we now have the opportunity to examine a virtually unexploited population. Provided the developing fishery is closely monitored, it should prove to be an ideal "testing ground" for further study.

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AASEN, O. 1964. The exploitation of the spiny dogfish (*Squalus acanthias* L.) in European waters. Fiskeridir. Skr. Fisk. 13(7): 5–16.

BARRACLOUGH, W. E. 1948a. The sunken gillnet fishery and an analysis of the availability of the dogfish (*Squalus suckleyi* Girard) and the soup-fin shark (*Galeorhinus galeus* Linnaeus in British Columbia waters from 1943 to 1946. M.A. Thesis, University of British Columbia, Vancouver, B.C. 139 p.

1948b. Measures of abundance in dogfish (*Squalus suckleyi*). Trans. R. Soc. Can. 42 (Ser. III) Sect. 5: 37–43.

BEAMISH, R. J., AND M. S. SMITH. 1976. A preliminary report on the distribution, abundance, and biology of juvenile spiny dogfish (*Squalus acanthias*) in the Strait of Georgia and their relationship with other fishes. Fish. Mar. Serv. Res. Dev. Tech. Rep. 629: 44 p.

BEVERTON, R. J. H., AND S. J. HOLT. 1960. A review of the lifespans and mortality rates of fish in nature and their relation to growth and other physiological characteristics. Ciba Found. Colloq. Ageing 5: 142–150.

BONHAM, K., F. B. SANFORD, W. CLEGG, AND G. C. BUCHER. 1949. Biological and vitamin A studies of dogfish landed in the State of Washington (*Squalus suckleyi*). Wash. Dep. Fish. Biol. Bull. 49A: 83–113.

CAUGHLEY, G. 1977. Analysis of vertebrate populations. John Wiley, Interscience, New York, N.Y. 234 p.

DELURY, D. B. 1947. On the estimation of biological populations. Biometrics 3: 145–167.

FORD, E. 1921. A contribution to our knowledge of the life-histories of the dogfishes landed at Plymouth. J. Mar. Biol. Assoc. U.K. N.S. 12: 468–505.

GULLAND, J. A. 1970. The fish resources of the ocean. FAO Fish. Pap. 97: 425 p.

HOLDEN, M. J. 1968. The rational exploitation of the Scottish–Norwegian stocks of spurdogs (*Squalus acanthias* L.). Min. Agr. Fish. Fd. (U.K.), Fish. Invest., Ser. II, 25 (8): 28 p.

1973. Are long-term sustainable fisheries for elasmobranchs possible? p. 360–367. *In* Fish stocks and recruitment, Rapp. P. V. Reun. Cons. Int. Explor. Mer. 164.

1974. Problems in the rational exploitation of elasmobranch populations and some suggested solutions, p. 117–137. *In* F. R. Harden-Jones [ed.], Sea fisheries research. Halsted Press, New York, N.Y. 510 p.

1977. Elasmobranchs, p. 187–214. *In* J. A. Gulland [ed.] Fish population dynamics, J. Wiley and Sons Ltd., London. 372 p.

JONES, B. C., AND G. H. GEEN. 1977a. Age determination of an elasmobranch (*Squalus acanthias*) by X-ray spectrometry. J. Fish. Res. Board Can. 34: 44–48.

1977b. Age and growth of spiny dogfish (*Squalus acanthias*) in the Strait of Georgia, British Columbia. Fish. Mar. Serv. Res. Dev. Tech. Rep. 699: 16 p.

1977c. Reproduction and embryonic development of spiny dogfish (*Squalus acanthias*) in the Strait of Georgia, British Columbia. J. Fish. Res. Board Can. 34: 1286–1292.

1977d. Food and feeding of spiny dogfish (*Squalus acanthias*) in British Columbia waters. J. Fish. Res. Board Can. 34: 2067–2078.

KETCHEN, K. S. 1969. A review of the dogfish problem off the west coast of Canada. Fish. Res. Board Can. MS Rep. Ser. 1048: 25 p.

1972. Size at maturity, fecundity, and embryonic growth of the spiny dogfish (*Squalus acanthias*) in British Columbia waters. J. Fish. Res. Board Can. 29: 1717–1723.

1975. Age and growth of dogfish, *Squalus acanthias*, in British Columbia waters. J. Fish. Res. Board Can. 32: 43–59.

LESLIE, P. H. 1945. On the use of matrices in certain population mathematics. Biometrika 33: 183–212.

LESLIE, P. H., AND D. H. S. DAVIS. 1939. An attempt to determine the absolute number of rats on a given area. J. Anim. Ecol. 8: 94–113.

SMITH, M. S. 1970. A. P. Knight groundfish cruise No. 69-1, December 2–11, 1969. Fish. Res. Board Can. MS Rep. Ser. 1078: 14 p.

1972. A. P. Knight groundfish cruise No. 71-1, November 18–25, 1971. Fish. Res. Board Can. MS Rep. Ser. 1164: 13 p.

1973. A. P. Knight groundfish cruise No. 72-1,

October 24–November 2, and November 23, 1972. Fish. Res. Board Can. MS Rep. Ser. 1233: 22 p.

1974. A. P. Knight groundfish cruise No. 73–2, October 22–30, 1973. Fish. Res. Board Can. MS Rep. Ser. No. 1290: 17 p.

1975. A. P. Knight groundfish cruise No. 74-2, October 15–17, 1974 (Data Record). Fish. Res. Board Can. MS Rep. Ser. 1336: 13 p.

SMITH, M. S., AND U. KRISTIANSEN. 1970a. A. P. Knight groundfish cruise No. 70-1, February 10–19, 1970. Fish. Res. Board Can. MS Rep. Ser. 1089: 21 p.

1970b. A. P. Knight groundfish cruise No. 70-2, July 20–23, 1970. Fish. Res. Board Can. MS Rep. Ser. 1106: 18 p.

SOUTHWARD, G. M. 1968. A simulation of management strategies in the Pacific halibut fishery. Int. Pac. Halibut Comm. Rep. 47: 70 p.

TROADEC, J. P. 1978. Semi-quantitative methods of stock assessment. *In* Models for fish stock assessment. FAO Fish. Circ. 701: 122 p.

WALTERS, C. J. 1969. A generalized computer simulation model for fish population studies. Trans. Am. Fish. Soc. 98: 505–512.

Phytoplankton Ecology of the Strait of Georgia, British Columbia

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STOCKNER, J. G., D. D. CLIFF, AND K. R. S. SHORTREED. 1979. Phytoplankton ecology of the Strait of Georgia, British Columbia. J. Fish. Res. Board Can. 36: 657–666.

Observations of phytoplankton production, abundance, and distribution were made at 16 stations in the Strait of Georgia from 1975 to 1977. The discharge of turbid Fraser River water exerts a strong influence on phytoplankton production and distribution in surface waters by rapid light attenuation and horizontal advection. At plume boundaries and back eddies where light conditions improve, very high production occurs (>4–5 g $C \cdot m^{-2} \cdot d^{-1}$), because of rapidly replenished nutrients supplied by the Fraser River. Advection, turbulence, zooplankton grazing, and summer nitrate depletion collectively impart a heterogeneous distribution pattern to phytoplankton in the surface waters of the Strait of Georgia. Mean annual production varies from lows of 150 g $C \cdot m^{-2}$ in Fraser River plume to highs of over 500 g $C \cdot m^{-2}$ in sheltered boundary waters of inlets. Recent increases in ammonia and nitrate from land drainage and domestic sewage, mainly through the Fraser River, are related to increases in phytoplankton standing stocks in the Strait.

Key words: phytoplankton, primary production, eutrophication, coastal marine, phytoplankton distribution and succession, chlorophyll *a*, pelagic

STOCKNER, J. G., D. D. CLIFF, AND K. R. S. SHORTREED. 1979. Phytoplankton ecology of the Strait of Georgia, British Columbia. J. Fish. Res. Board Can. 36: 657–666.

De 1975 à 1977, on a fait des observations sur la production, l'abondance et la distribution du phytoplancton à seize stations dans le détroit de Géorgie. Le débit d'eau turbide du fleuve Fraser influe fortement sur la production et la distribution du phytoplancton en surface par le biais d'une rapide atténuation de la lumière et de l'advection horizontale. À la limite des couches superficielles et des remous où les conditions lumineuses sont meilleures, il y a très forte production (>4–5 g $C \cdot m^{-2} \cdot d^{-1}$), par suite du renouvellement rapide des éléments nutritifs fournis par le fleuve Fraser. Le phytoplancton des eaux superficielles du détroit de Géorgie doit sa distribution hétérogène à l'advection, la turbulence, le broutage par le zooplancton et l'épuisement estival des nitrates. La production annuelle moyenne varie de creux de 150 g $C \cdot m^{-2}$ dans la couche superficielle du fleuve Fraser à des pics dépassant 500 g $C \cdot m^{-2}$ dans les couches limites abritées des bras de mer. Il existe une relation entre les récentes augmentations d'ammoniac et de nitrates provenant du ruissellement et les égouts domestiques, surtout par le fleuve Fraser, et l'accroissement de la biomasse phytoplanctonique dans le Détroit.

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THE whole of the Strait of Georgia is essentially an estuary because it has free access to the open ocean and its surface waters are measurably diluted with fresh water from land drainage. The Strait of Georgia is extremely important as a nursery and rearing ground for valuable Pacific salmon and herring fisheries. It also provides a lesser fishery for cod, groundfish, shellfish, shrimps, and crabs.

There have been many studies of the Strait of Georgia that span nearly 70 yr of observation. Among the notable physical and chemical oceanographic works are those by Tully and Dodimead (1957), Waldichuk (1957), and Waldichuk et al. (1968). Plankton studies have dominated the biological literature over the decades and include the descriptive works of Hutchinson and Lucas (1931), Légaré (1957), and the more theoretical treatments by Parsons et al. (1969a, b), Parsons and LeBrasseur (1970), and Takahashi et al. (1973). The majority of these studies are limited because they deal either with specific regions, i.e. Fraser River plume, or they lack some continuity because of a paucity of observations over the entire area for several consecutive seasons.

The primary objectives of this study were (1) to describe over two growing seasons the wax and wane of phytoplankton populations, including their production, distribution, and seasonal succession; (2) to assess, where possible, the impact of natural and man-induced stress on phytoplankton; and (3) to compare our recent annual and daily primary production rates with earlier published values from the strait and with world

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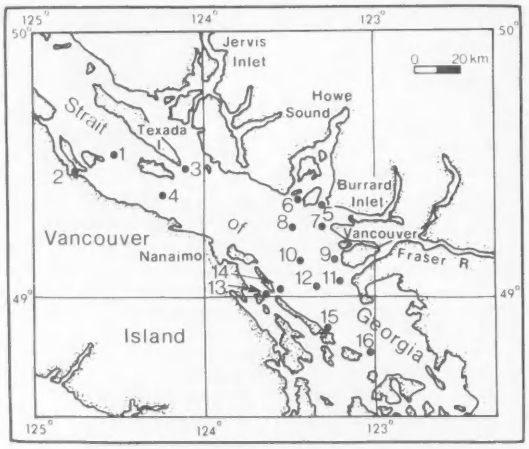

FIG. 1. Strait of Georgia showing station locations and major geographic features.

oceanic and coastal marine values, so as to place in perspective the production potential of the Strait of Georgia.

Description of Study Area

The Strait of Georgia is a complex marine waterway bounded on the west by Vancouver Island, on the east by mainland British Columbia, and on the south by Washington State (Fig. 1). Its length is 200 km, average width 33 km, and mean depth 156 m. Passages to the Pacific Ocean are generally narrow and restrict tidal flow, resulting in extreme tidal turbulence.

Major factors influencing hydrography of the area are fresh water runoff, tide, wind, and insolation. The turbid Fraser River water contributes ~80% of the total land runoff and causes most of the salinity variation in surface waters. Tides are mixed semidiurnal with a maximum daily range of 5 m. Precipitation ranges from 90 cm·yr^{-1} in parts of the southern Strait of Georgia to over 200 cm·yr^{-1} near the British Columbia mainland in the north. Pritchard's (1967) definition classifies the Strait of Georgia as a positive estuary, since precipitation and runoff exceed evaporation, and net flow is seaward. Prevailing winds are SE during winter and NW in summer, mean air temperatures are 2°C in winter and 18°C in summer, and annual insolation ranges from 2163 h in the south to 1489 h in the north near the B.C. mainland (Waldichuk 1957).

The strait may be divided into three oceanographic areas:

1) The stable main body where distinct stratification occurs, and tidal currents rarely exceed 2–4 km·h^{-1}.

2) Areas of turbulent tidal mixing where currents may exceed 9 km·h^{-1} and stratification is indistinct (near north and south passages to the Pacific Ocean and in the narrow Gulf Island passes).

3) The area contiguous to the Fraser Delta and overlain by the turbid Fraser River plume, whose total area is highly variable and dependent on river discharge, wind, and tide.

Materials and Methods

Total incident solar radiation was recorded on a Belfort pyrheliometer mounted on board ship, and extinction of surface light with depth was measured by a Montedoro–Whitney Underwater Illuminance meter (Model LMT-8A). The natural logarithm of light intensity was plotted against depth, and the slope of a straight line fitted to the points by linear regression provided an estimate of the mean downward extinction coefficient (k). A standard 30-cm white Secchi disc was used to measure water transparency at every station.

Temperature profiles to a depth of 50 m were obtained with a bathythermograph and surface temperatures were measured with a bucket thermometer.

Water samples were collected from 1, 3, 5, and 20 m depths, and later analyzed for carbonate alkalinity, salinity, dissolved oxygen, nitrate (NO_3^{-1}), total phosphate (TPO_4^{-3}), and silicate (SiO_4^{-4}). The method of Strickland and Parsons (1968) was used to determine carbonate alkalinity, using an Orion digital pH meter (Model 801). However, if after acid titration final pH values fell below 3.00, then the sample analysis was repeated using the APHA et al. (1971) standard titrimetric method. Salinity was analyzed with an Auto-lab salinometer (Model 601) to an accuracy of ±0.5‰. Density was computed by nomograph from temperature and salinity records. Dissolved oxygen (samples fixed within an hour of being taken) was determined by use of the Winkler titration method (Strickland and Parsons 1968). Samples for nutrient analysis were immediately frozen and later analyzed by the Environmental Protection Service analytical laboratory, Pacific Environment Institute, West Vancouver, using methods outlined in their manual (Fisheries/EPS 1974).

The standard ^{14}C method as initially proposed by Steemann-Nielsen (1952) was used, with some modifications. Water was collected from surface skim, and 0.5, 1, 2, 3, 5, 10, 20, and 30 m depths using a 6-L polyvinylchloride Van Dorn bottle. Productivity bottles (two 125 mL light and one 125 mL dark) were inoculated with 1 mL $NaH^{14}CO_3$ (37 kBq·mL^{-1}). For each experiment, the number of disintegrations·min^{-1} (dpm)·mL^{-1} was determined by placing 1 mL in each of three scintillation vials. In most cases, samples were incubated in situ for 4–5 h, normally from 0930 to 1430. Water was filtered through 0.45 μm BDH cellulose nitrate filters and placed in 10 mL of a specially prepared toluene-based fluor (POPOP, PPO, 2-ethoxyethanol, and toluene). Samples were analyzed within 24 h for activity in a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3375). The equation of Strickland (1960) was used to convert dpms to mg C·m^{-3}. Using incident solar radiation for the entire light day and for the incubation period, data were converted to mg C·m^{-3}·d^{-1} and integrated by a Hewlett–Packard Calculator and Plotter (Model 9820 A, 9862 A) to give phytoplankton production on an area basis (mg C·m^{-2}·d^{-1}). This involved linear interpolation between data points to the depths where light and dark uptake were equal.

Detailed examination was made of phytoplankton samples from only 1 and 5 m depths to assess (a) species composition and (b) phytoplankton volumes during key spring and fall bloom periods. Phytoplankton samples were preserved in Lugol's acetate solution and enumerated, using the Utermöhl (1958) sedimentation method. Volumes were determined by equating phytoplankton cells to known geo-

metric shapes. Each phytoplankton sample was examined under 175 and 700× magnification using a Wild M40 inverted plankton microscope. Results were expressed as cells and total volume $\cdot m^{-3}$.

One liter of sea water was taken for chlorophyll *a* analysis from 1, 3, 5, and 20 m depths and filtered onto 5.5-cm Whatman GF/C glass fiber filters with a small amount of $MgCO_3$ added. Filters were macerated in a tissue grinder with 10 mL of 90% acetone, and the filtrate analyzed on a Cary recording spectrophotometer (Model 15). The equation of Strickland and Parsons (1968) was used to calculate chlorophyll *a*.

Vertical zooplankton hauls from 50 m were made at each station with a SCOR–UNESCO plankton net, with mouth diameter of 57 cm and screen mesh size of 350 μm. Samples were preserved in 5% formaldehyde, returned to the laboratory, and split into two equal subsamples. One portion was dried overnight at 90°C, and results expressed as zooplankton biomass in mg (dry weight) $\cdot m^{-3}$. The other portion was preserved for future reference.

Results

Physics and Chemistry

Thermal stratification developed by April and broke down in October except in areas of strong tidal flow, where stratification was transitory or entirely lacking. Surface temperatures ranged from 4°C in winter to 18°C in summer (Table 1). In turbulent passages, surface temperatures varied with each tidal cycle (Tully and Dodimead 1957).

Surface layer salinities ranged from 0.5‰ near the Fraser River during spring freshet to 30‰ away from fresh water influences in winter. Deep-water salinities varied little and were rarely <28‰. A distinct halocline existed in all but the winter months, except near the turbulent passes, where well-mixed water prevented its development.

The pycnocline depth ranged from 5 to 14 m in the Strait of Georgia, with surface σ_T values ranging from 5 to 24. Deep waters varied little around a mean of 22 (Table 1). The pycnocline disappeared in the turbulent passages.

Dissolved oxygen ranged seasonally from 4 to 10 mg $\cdot L^{-1}$ over the whole water column. Highest values occurred at or just below the surface during the vernal phytoplankton bloom.

Ranges of nitrate-N, total phosphate-P, and silicate-Si in Georgia Strait were <0.01–0.47 mg $\cdot L^{-1}$ (<0.7–33 μg-atoms $\cdot L^{-1}$), <0.01–0.19 (<0.3–5.9), and <0.50–3.40 (<17.8–121.4), respectively. Nitrate depletion in the surface layer occurred in areas and times of stable stratification, usually during July, August, and September. Highest mean values of nitrate occurred at Stations 15 and 16 contiguous to turbulent passages (Table 1). At Station 16 in the turbulent Haro Strait the mean TPO_4 value was higher than that recorded at other stations in the strait.

Mean extinction coefficients (k) ranged from 0.11 in winter to 2.90 near the Fraser River during the spring freshet.

Plankton

By early April, sufficient light ($\bar{x}$ >502 J $\cdot cm^{-2}$ d^{-1}) and surface layer stability occurred to permit substantial increases in phytoplankton standing stock. The increases were noticeable first in the more sheltered and protected waters of the southern strait, but with increasing vertical stability, phytoplankton increased in northern sectors of the strait, where peaks occurred in late May or early June. This phase lag between north and south was due largely to differences in vertical stability among regions of the strait (Parsons 1965).

At all stations except those in areas of strong tidal flow, the spring phytoplankton bloom resulted in maxima of chlorophyll *a*, primary production, and phytoplankton numbers and volume. In areas of strong tidal flow, these factors showed only slight seasonal increases (Fig. 2). Fall increases of chlorophyll and primary production occurred at stratified and stable stations, but a distinct bimodal pattern was not apparent at stations in exposed or tidally mixed areas.

Definite seasonal trends in phytoplankton species succession for the Strait of Georgia were not apparent in this study, as variability among stations made generalization difficult (Fig. 3A, B). Diatoms, mainly *Chaetoceros* spp., *Thalassiosira* spp., *Skeletonema costatum*, *Coscinodiscus* spp., and *Cerataulina bergonii*, were the dominant group throughout the Strait of Georgia in spring and fall periods. At times in August the dinoflagellates *Peridinium* spp., *Gymnodinium* spp., and *Dinophysis* spp. were also common. Other common phytoplankters were unidentified flagellates, occurring over a wide size range and the silicoflagellates *Ebria tripartita* and *Distephana speculum*.

Mean annual chlorophyll *a* and mean annual production were highest in the sheltered waterways of the Gulf Islands (Station 13, Fig. 4), and lowest in areas of strong tidal flow (Station 16), and at stations near the discharge of the turbid Fraser River, where shifting plume boundaries and rapidly changing light conditions affected production (Station 11). Annual production ranged from 149 to 511 g C $\cdot m^{-2} \cdot yr^{-1}$, integral chlorophyll *a* from 1.0 mg $\cdot m^{-2}$ at some stations in winter to 480 during the vernal bloom, mean phytoplankton numbers from 4.00×10^6 to $1.79 \times 10^{10} \cdot m^{-3}$, and mean phytoplankton volume from 1.00 $mm^3 \cdot m^{-3}$ to 3.46×10^4 $mm^3 \cdot m^{-3}$ (Table 1 and Fig. 5).

Mean zooplankton biomass among stations ranged from <1 to 320 mg $\cdot m^{-3}$, with lowest values recorded in winter and highest in spring, with a phase lag in peak density occurring ~1 mo after the vernal phytoplankton bloom (Stockner et al. 1977). Lowest mean annual zooplankton biomass in the Strait of Georgia occurred at Station 1, while the highest was recorded at Station 3 in the northern strait off the tip of Texada Island (Table 1).

TABLE 1. Maximum–minimum values and 2-yr means of selected physical, chemical, and biological variables in the surface layer (0–5 m) of the Strait of Georgia in 1976 and 1977.

Station	Temp (°C)	Salinity (‰)	Density (σ_T)	Mixed layer depth (m)	Dissolved oxygen (mg·L^{-1})	NO_3-N (mg·L^{-1})	Total PO_4-P (mg·L^{-1})	SiO_4 as Si (mg·L^{-1})	Extinction coeff. (k)	Secchi depth (m)	Chl. a $\int_0^{25m}$ (mg·m^{-2})	Primary production g C·m^{-2}·yr^{-1}	Algal no. no.×10^6·m^{-3}	Algal vol (mm^3·m^{-3})	Zooplankton biomass (mg·m^{-3})
1	7.3–18.6 11.7	25.5–29.6 28.1	17.9–22.9 21.2	8.3	5.0–7.3 6.5	0.02–0.47 0.21	0.03–0.09 0.05	0.50–1.70 0.86	0.17–0.58 0.29	7.0–10.0 8.3	9.7–106.7 35.5	216	12–6013 1954	6–3047 651	4.3–16.3 8.9
2	7.5–17.4 11.4	26.6–29.4 28.2	19.0–22.7 21.4	13.5	5.0–7.0 6.3	0.01–0.40 0.20	0.02–0.08 0.05	0.50–1.65 0.83	0.17–0.35 0.28	6.5–16.0 9.0	12.2–149.2 61.0	465	69–3936 1872	62–14829 2605	1.9–18.8 10.8
3	6.2–18.9 11.7	12.9–29.4 24.6	11.7–22.7 19.1	11.8	5.8–10.0 7.4	0.01–0.38 0.13	0.02–0.12 0.05	0.50–2.25 1.07	0.11–0.37 0.24	4.3–12.0 8.0	6.6–113.4 31.4	342	31–4968 1216	26–6015 951	0.4–315.2 59.8
4	6.6–16.5 11.2	11.7–29.2 25.3	11.8–22.8 19.6	12.3	6.4–9.4 7.2	0.01–0.35 0.14	0.02–0.14 0.05	0.50–2.28 0.99	0.11–0.46 0.25	3.5–12.0 8.8	8.5–183.2 48.9	455	4–5459 1511	2–12055 2242	1.5–192.9 34.9
5	6.0–18.5 10.8	10.2–29.6 22.2	6.4–23.2 16.7	7.7	5.0–10.0 7.0	0.01–0.39 0.14	0.01–0.10 0.05	0.50–2.75 1.05	0.16–1.33 0.42	1.3–8.0 4.6	<1.0–460.4 54.7	374	8–17916 3159	1–27218 5203	0.8–157.3 19.1
6	5.5–18.8 10.9	13.9–28.9 22.6	9.2–22.6 17.4	8.2	5.2–10.0 7.1	0.01–0.37 0.13	0.01–0.14 0.04	0.41–2.80 0.94	0.15–0.94 0.39	2.3–8.5 5.7	<1.0–479.3 46.2	292	24–13315 2506	6–17538 3863	1.2–319.8 22.1
7	5.7–17.0 11.0	7.8–29.4 21.1	5.0–22.8 16.1	6.8	4.5–8.6 6.9	0.01–0.30 0.13	0.02–0.17 0.05	0.50–3.20 1.05	0.21–1.54 0.55	0.3–10.5 3.1	4.3–209.2 46.5	338	28–10878 2124	35–32512 3109	1.8–116.4 20.4
8	5.6–18.7 11.1	9.1–28.1 23.0	6.0–21.9 17.4	8.6	6.4–8.0 7.2	0.01–0.35 0.12	0.01–0.14 0.05	0.50–2.25 1.00	0.13–0.73 0.37	0.3–13.0 5.2	2.0–270.2 51.5	349	12–11035 2026	2–21414 2751	1.7–316.6 42.2
9	6.2–14.8 10.7	20.5–27.1 24.1	15.2–21.0 18.2	4.0	5.9–8.2 7.1	0.02–0.32 0.12	0.02–0.18 0.08	0.50–1.65 0.92	0.21–2.90 0.79	1.0–5.0 2.3	9.7–105.5 40.7	297	46–8634 3079	28–34637 5103	7.2–42.8 17.5
10	6.3–15.7 11.3	19.2–27.2 24.1	14.0–21.4 18.3	7.0	6.4–9.0 7.5	0.03–0.37 0.12	0.01–0.16 0.07	0.50–1.50 0.81	0.14–1.15 0.48	2.5–7.0 4.5	14.5–202.9 56.0	401	75–9210 3226	13–32494 5035	2.7–122.6 30.5
11	5.1–18.0 11.2	11.1–28.3 20.4	8.1–22.3 15.4	5.6	5.7–8.1 6.8	0.01–0.30 0.13	0.01–0.07 0.05	0.50–2.80 1.23	0.21–1.96 0.73	0.5–6.5 2.8	3.9–424.7 43.1	149	20–6855 1374	3–26892 1785	3.2–298.1 46.7
12	5.9–15.8 11.1	10.9–28.4 22.0	7.6–22.1 16.4	8.0	6.2–8.7 7.0	0.01–0.30 0.13	0.01–0.07 0.05	0.50–3.40 1.23	0.18–1.31 0.42	0.8–7.0 3.2	3.1–254.3 50.6	262	8–10279 1817	6–18522 2498	1.5–232.8 35.0
13	6.8–15.0 10.2	24.8–29.0 27.6	18.8–22.8 21.2	6.0	4.5–7.4 6.4	0.04–0.34 0.19	0.04–0.10 0.07	0.50–2.00 1.09	0.17–0.38 0.26	4.0–11.0 6.9	8.0–156.4 65.3	511	14–4121 1558	34–11444 2550	2.9–70.6 16.8
14	6.9–15.8 10.3	16.2–29.1 26.4	11.9–22.9 20.5	7.7	4.6–7.8 6.5	0.01–0.37 0.22	0.02–0.13 0.06	0.50–1.75 1.01	0.17–0.53 0.28	2.5–11.5 7.6	4.2–136.4 42.7	377	4–4950 1334	6–16812 2314	2.0–74.1 21.8
15	6.4–19.2 10.6	19.5–29.6 25.7	13.2–23.8 19.5	8.0	4.7–7.8 6.8	0.01–0.38 0.20	0.02–0.13 0.06	0.50–1.92 0.98	0.16–0.36 0.24	2.5–9.0 6.7	8.0–156.4 40.9	397	12–4205 1717	12–17595 2654	2.9–70.6 28.7
16	7.0–14.3 9.4	28.0–30.5 29.5	21.3–23.8 22.8	un- stable	4.5–7.3 5.7	0.16–0.40 0.29	0.05–0.19 0.08	0.50–2.10 1.11	0.17–0.43 0.26	3.8–9.5 7.2	4.5–93.6 28.5	289	8–4213 1385	4–4771 1605	4.0–31.0 12.8

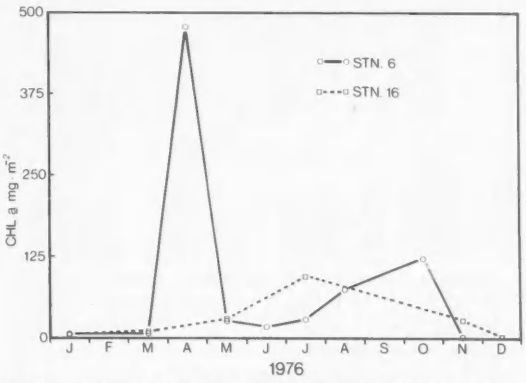

FIG. 2. Seasonal variation in chlorophyll *a* in the surface layer (0–20 m) at Station 6 in boundary waters of Howe Sound and Station 16 in the turbulent Haro Strait.

Discussion

The oceanography of the Strait of Georgia is to a large extent dominated by the inflow of the Fraser River, which imparts turbidity to the surface layer and markedly affects surface layer stability and salinity. Past physical studies have demonstrated the general northward movement of the water mass from off the Fraser delta, which influences the surface layer of the north mainland coast and sets up a counterclockwise gyre in the western portion of the southern strait (Waldichuk 1957). This generalized physical transport system strongly influences the distribution and production of phytoplankton.

The impact of the Fraser River discharge on phytoplankton growth in the southern strait is illustrated in Fig. 6, where mean 1976 values of light, chlorophyll *a*, production, and P:B (photosynthesis/chlorophyll) ratios at stations along a transect from the south Fraser mouth to inside Porlier Pass are plotted. Light attenuation is one of the major factors influencing primary production from June to September in surface waters of the strait affected by the Fraser River discharge, as can be seen by the linear decline of the extinction coefficient (k), from the turbid delta front to the arbitrary limits of the plume, and its inverse relation to phytoplankton production and biomass (Fig. 6). At Station 10, which was usually sampled at the boundary of the Fraser Plume, using stepwise multiple linear regression it was clear that throughout the growing season NO_3, light (k), and zooplankton grazing were the factors, listed in descending order of importance, limiting the production of organic carbon ($R = .90$ df 4 and 13, $F = 29.73$, $P < 0.05$).

The primary factors limiting production in areas of the Strait of Georgia not markedly affected by the Fraser River (Station 3) are in descending order of importance: grazing, NO_3, and light ($R = .88$, df 4 and 8, $F = 15.36$, $P \leq 0.05$). Earlier work by Strickland

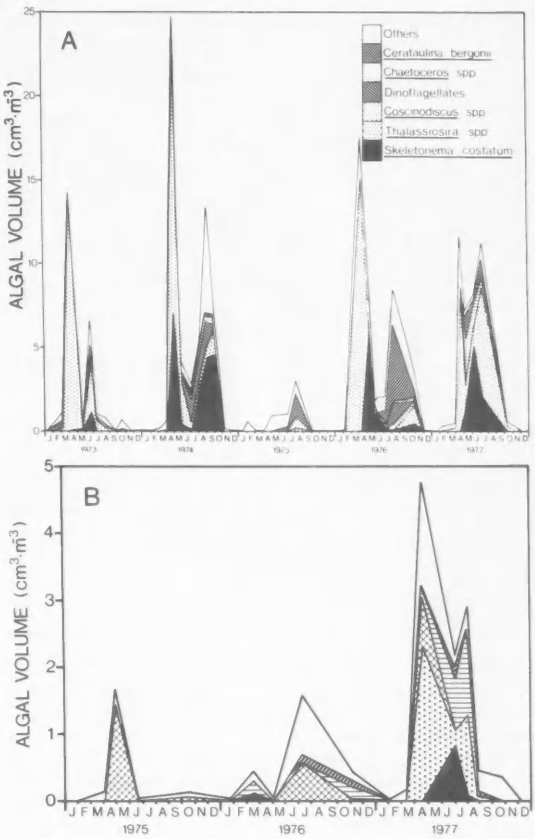

FIG. 3. A, Five-year seasonal succession of phytoplankton in the strait at Station 6 in the boundary waters of Howe Sound; B, Three-year seasonal succession of phytoplankton at Station 16 in the turbulent Haro Strait. Species shown at both stations are those contributing to >5% of the total phytoplankton volume.

(1958), Antia et al. (1963), and Parsons et al. (1969a) has demonstrated nitrogen limitation in June and early July in the strait, and concluded that because of its relatively slow regeneration rate, it is the limiting nutrient in most situations. Silicate levels are low in June and July following the vernal diatom increases, but never as low as values reported by Paasche (1973) to be limiting to marine diatoms.

Though zooplankters were not studied in any detail, seasonal trends in biomass were apparent at most stations, showing a phase lag with phytoplankton peaks of about a month, and our statistical evaluation of the factors influencing primary production consistently underscored the significance of grazing on the accumulation of organic matter (chlorophyll *a*) in the strait. Gardner's (1977) recent studies have shown a

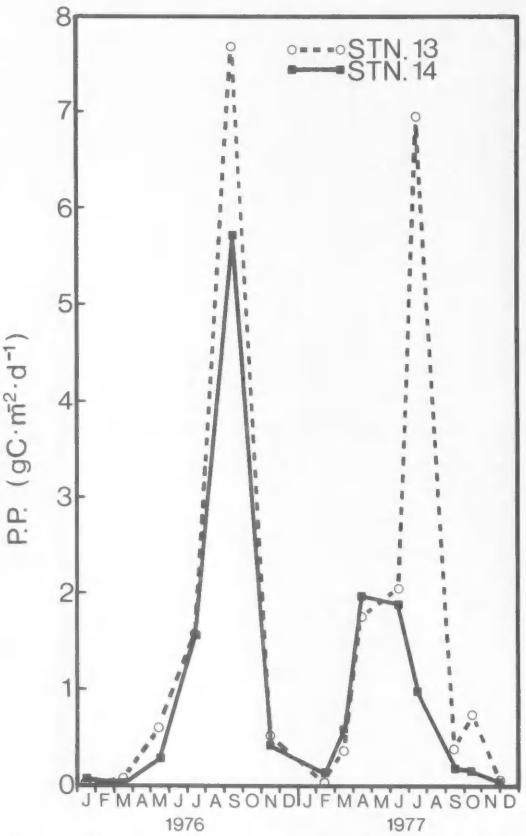

FIG. 4. Seasonal changes in primary production at Station 13 located in the sheltered waters of Trincomali Channel inside Porlier Pass and at Station 14 in the open strait outside Porlier Pass in 1976 and 1977.

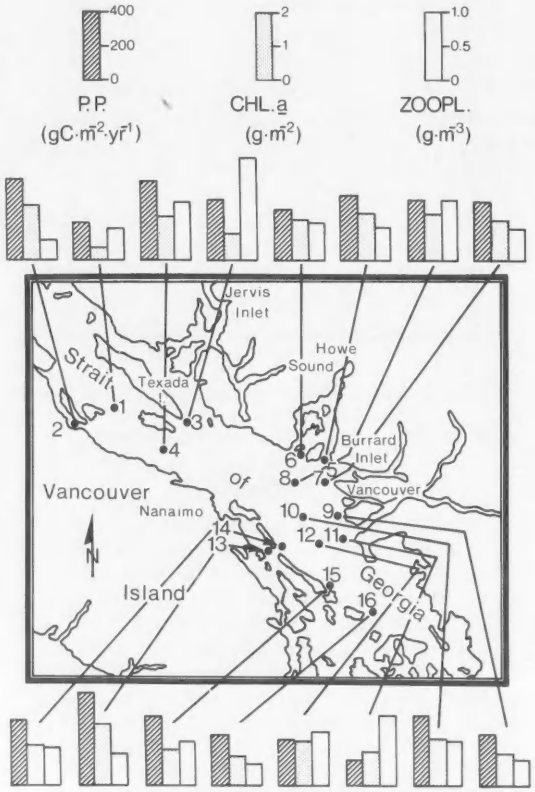

FIG. 5. Mean annual integrals of phytoplankton production, chlorophyll *a*, and zooplankton biomass by station in the Strait of Georgia.

significant relation between late summer hydrographic conditions (temperature and mixed-layer stability) and overwintering zooplankton populations. Conditions in the Strait of Georgia in the fall and winter of 1974 led to a large overwintering population that prevented any significant development of the vernal phytoplankton bloom in 1975 in some regions of the strait as typified by observations from Station 6 (Fig. 7).

The predominant northward movement of the Fraser River plume has increased production in boundary waters of Howe Sound, a region of the strait whose waters are not strongly influenced by Fraser River turbidity, yet receive by advection phytoplankton from the productive boundary waters and benefit from nutrient "recharge" supplied by periodic episodes of deep mixing in summer months (Stockner et al. 1977) (Fig. 8). In regions less affected by the Fraser River discharge, notably the mid- and northwestern regions of the strait, annual production is lower, ranging from 200 to 300 g C·m^{-2}. The incoming colder and more saline oceanic water, which creates vertical instability at turbulent passages, reduces phytoplankton production in many regions of the American San Juan Islands and lower Gulf Islands (Phifer 1933; Phifer and Thompson 1937), and to the north in Johnstone Strait (Stockner and Cliff 1976a) (Fig. 8).

Our mean annual estimates of production for the Strait of Georgia are very similar to recent estimates by Winter et al. (1975) of 465 g C·m^{-2}·yr^{-1} for the central basin of Puget Sound in Washington State, and to estimates by Malone (1976) and Mandelli et al. (1970) of 370 and 420 g C·m^{-2} for the apex of the New York Bight, and by Riley (1956) (380 g C·m^{-2}) for Long Island Sound, but substantially higher than open ocean estimates (Ryther and Yentsch 1958; Taniguchi 1972), ranging from 80 to 160 g C·m^{-2}.

Parsons et al. (1970), from values obtained from shipboard incubation of five depths (0, 5, 10, 20,

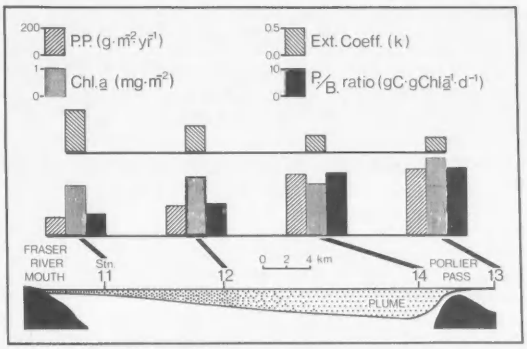

FIG. 6. Schematic illustration of the impact of the Fraser discharge on light attenuation and primary production in the Straight of Georgia. Histograms are mean 1976 values from stations on an east–west transect from river mouth to inside Porlier Pass.

and 30 m) at eight stations, estimated annual production for the Strait of Georgia to average 120 g C·m^{-2} in 1965 and 1966, which is much lower than our average of 345 g C·m^{-2} obtained in situ at nine depths from 16 stations. By recalculating our monthly integral production values using the five depths reported by Parsons et al. (1970), we could account for an average loss of from 5 to 30% depending on the month of observation (small error in winter, high in summer). Therefore methodological differences alone cannot account for the observed differences in annual production between 1966 and 1976–77. Using chlorophyll *a* values obtained by Stephens (1968) from his station 7, which corresponds to our Station 10, we integrated values from 1, 3, and 5 m depths for the years 1965 and 1977 and found mean amounts of chlorophyll in 1977 to be 1.6× those reported from 1965, 2.2 and 3.5 mg·m^{-3}, respectively. This comparison, despite the uncertainties attributable to yearly variation, supports the hypothesis

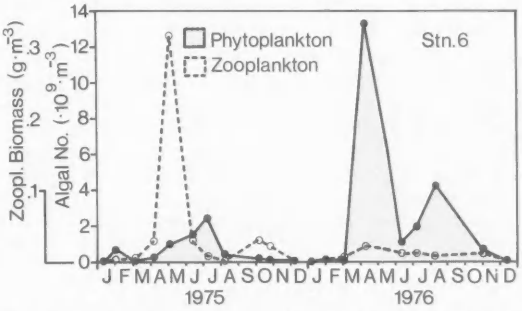

FIG. 7. Seasonal changes in the abundance of phytoplankton and zooplankton at Station 6 in the boundary waters of Howe Sound in 1975 and 1976.

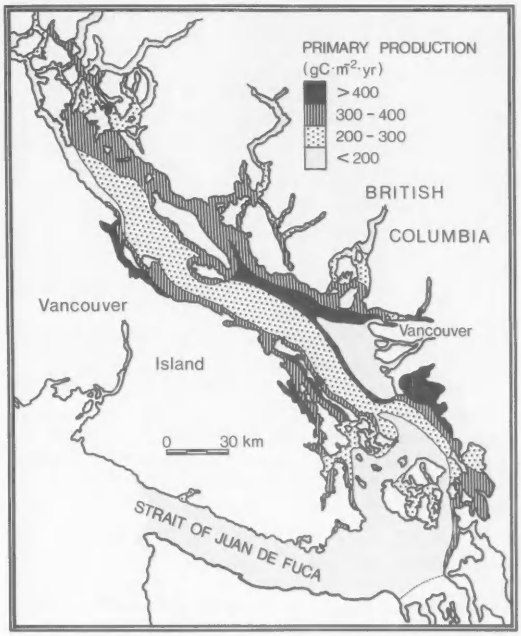

FIG. 8. Generalized spatial pattern of annual phytoplankton production in the Strait of Georgia. Data used in preparation of figure represent 5 yr of plankton studies by J. G. S. (Stockner and Cliff 1975, 1976a, 1976b; Stockner et al. 1977) and data presented by Waldichuk (1957), Waldichuk et al. (1968), Stephens (1968), Barraclough and Fulton (1967), Stephens et al. (1969), and Parsons et al. (1970).

that primary production in the strait has increased over the past 10 yr. We feel that these increases are due in part to eutrophication.

Based on per capita contributions, a minimal estimate of the nitrogen (N) and phosphorus (P) loading to the Strait of Georgia in 1976 from the metropolitan area of Vancouver and the Fraser River drainage basin was ~15 000 t of N and 3500 of P (Table 2). The loading has increased markedly over the past two decades, owing to the rapid population growth in the lower mainland of British Columbia. Perhaps even more significant is the fact that the nutrient discharge points to the strait have changed markedly since 1974. In the 1950s and 1960s most nutrients derived from sewage from metropolitan Vancouver were discharged to the Burrard Inlet system (Vancouver Harbor and English Bay), but by 1974 almost all had been diverted to the Fraser River (S. Vernon, Greater Vancouver Regional District, personal communication). This has likely improved conditions in Vancouver Harbor (Stockner and Cliff 1979), and increased production in back eddies and plume boundaries of the Fraser River

TABLE 2. Nitrogen and phosphorus loadings to the Strait of Georgia by decade from 1951 to 1977.[a]

Year	Population[d]	Industrial[e]		Storm sewer[e]		Domestic[e]		Total[b]		Loading[c] g·m^{-2}·yr^{-1}	
		N*	P	N	P	N	P	N	P	N	P
1951	649 200	753	280	202	64	4 300	876	6 832	1 586	0.99	0.23
1961	907 600	1 052	392	283	89	6 012	1 226	9 036	2 099	1.33	0.30
1971	1 156 505	1 340	500	361	114	7 658	1 562	12 167	2 829	1.76	0.41
1977	1 515 000	1 680	627	453	143	9 603	1 960	15 257	3 549	2.21	0.51

[a]All values in metric tons per year unless otherwise indicated.
[b]Estimates of N and P loading to entire strait increased 23% to account for loadings from other major river systems discharging to the strait (Waldichuk 1957).
[c]Surface area: 6900 km^2 (Waldichuk 1957).
[d]Statistics Canada census figures.
[e]Values from Dr K. Hall, Westwater Research Center, U.B.C., Vancouver, B.C.

(Fig. 8). Evidence to suggest that this has in fact occurred is provided by a comparison of the 1967 annual production value published by Takahashi et al. (1973) from their plume station with our 1977 value from Station 9 located at approximately the same position in the plume. Weather records indicate that 1967 and 1977 were similar years, yet mean primary production has increased by about a factor of 4, from 72 to 280 g C·m^{-2}·yr^{-1}, while conservative estimates of nitrate loadings have increased by ~30–40% since 1967 (Table 2).

Tully and Dodimead (1957) and Parsons et al. (1970), from studies in the 1950s and 1960s, suggested that nutrients in the surface layer of the Fraser River plume come largely from entrainment of nutrient-rich sea water, while our data suggest that nutrients necessary to support the magnitude of phytoplankton growth *now* seen in back eddies and plume boundaries may largely be derived from nitrate and ammonia from the Fraser River and adjoining tidal lands, and secondarily from sea water by physical processes. Nitrate is the limiting nutrient in the strait (Antia et al. 1963), and is rapidly incorporated into phytoplankton biomass and thus is not reflected in a concentration increase. Mean values of NO_3(N) and NH_3(N) in Fraser River water at Tilbury Island, several kilometres above the delta front, in 1976 were 154 and 43 μg·L^{-1} (11 and 3 μg-atoms·L^{-1}), respectively (K. J. Hall unpublished data). Earlier values from the 1960s are sparse, but the average nitrate concentration was considerably less, ~72 μg·L^{-1} (ammonia was not measured) (Benedict et al. 1973). Clearly, the concentration of nutrients in the Fraser River has increased, and accordingly the rate of primary production in areas influenced by it. It is important to note that these comparisons pertain *only* to dissolved inorganic nitrogen and recent evidence points to the importance of dissolved organic nitrogen (DON) in regulating phytoplankton productivity and succession in the sea (Butler et al. 1979).

Takahashi et al. (1973) reported nitrate limitation of phytoplankton growth during summer 1967 at their plume station; values from 1975 to 1977 from Stations 7, 9, and 11 in the plume in summer also reached undetectable levels, but our production estimates in back eddies off the delta front in August often were in excess of 4 g C·m^{-2}·d^{-1}, and this production occurred over the depth integral 0–4 m. The highest daily value recorded by Parsons et al. (1969b) from 0 to 30-m profile was 2.3 g C·m^{-2} in April 1967 at their Fraser River plume station.

The impact of the Fraser River water on phytoplankton production in the Strait of Georgia is very similar both in process and impact to that described for the New York Harbor (Mandelli et al. 1970; Ryther and Dunstan 1971) and apex of the Bight (Segar and Berberian 1976; Malone 1976), where ammonia and nitrate from land drainage and domestic sources in the low salinity surface outflow were directly related to the eutrophication of this large coastal marine environment.

The present levels of production in the Strait of Georgia can only be viewed as a positive benefit to the fisheries, where there are large resident populations of coho and chinook salmon and herring, and where millions of transitory juvenile salmonids feed on their way to the northwest Pacific Ocean (Argue 1976). Future enhancement of salmonid stocks by hatcheries and other enhancement techniques in British Columbia's lower mainland could conceivably tax the carrying capacity of the Strait of Georgia unless future consideration is given to studies of factors limiting production of pelagic communities in the coastal marine environment of the northwest Pacific ocean.

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AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1971. Standard methods for the examination of waste and waste-water. 13th ed. Washington, D.C. 769 p.

ANTIA, N. J., C. D. MCALLISTER, T. R. PARSONS, K. STEPHENS, AND J. D. H. STRICKLAND. 1963. Further measurements of primary production using a large-volume plastic sphere. Limnol. Oceanogr. 8: 166–183.

ARGUE, A. W. 1976. Preliminary information from the 1973 and 1974 Canadian chinook and coho catch sampling and mark-recovery program. Fish. Mar. Serv. Tech. Rep. (Pac. Region) PAC-T-76-9: 32 p.

BARRACLOUGH, W. E., AND J. D. FULTON. 1967. Data record. Number, size composition and food of larval and juvenile fish caught with a two-boat surface trawl in the Strait of Georgia, July 4–8, 1966. Fish. Res. Board Can. MS Rep. 940: 82 p.

BENEDICT, A. H., K. J. HALL, AND F. A. COCH. 1973. A preliminary water quality survey of the lower Fraser River system. Westwater Res. Center, Univ. B.C. Tech. Rep. 2: 50 p.

BUTLER, E. I., S. KNOX, AND M. I. LIDDICOAT. 1979. The relationship between inorganic and organic nutrients in sea water. J. Mar. Biol. Ass. U.K. 59: 239–250.

FISHERIES/ENVIRONMENTAL PROTECTION SERVICE. 1974. Laboratory manual. Dep. Fish. Environ. Fish. Mar. Serv. Pac. Reg. 180 p.

GARDNER, G. A. 1977. Analysis of zooplankton population fluctuations in the Strait of Georgia, British Columbia. J. Fish. Res. Board Can. 34: 1196–1206.

HUTCHINSON, A. H., AND C. C. LUCAS. 1931. The epithalassa of the Strait of Georgia. Can. J. Res. 5: 231–284.

LÉGARÉ, J. E. H. 1957. The qualitative and quantitative distribution of plankton in the Strait of Georgia in relation to certain oceanographic factors. J. Fish. Res. Board Can. 14: 521–552.

MALONE, T. C. 1976. Phytoplankton productivity in the apex of the New York Bight: Environmental regulation of productivity/chlorophyll *a*. Am. Soc. Limnol. Oceanogr. Spec. Symp. 2: 260–272.

MANDELLI, E. P., P. R. BURKHOLDER, T. E. DOHENY, AND R. BRADY. 1970. Studies of primary productivity in coastal waters of southern Long Island, New York. Mar. Biol. 7: 156–160.

PAASCHE, E. 1973. Silicon and the ecology of marine plankton diatoms. I. *Thalassiosira pseudonana* (*Cyclotella nana*) grown in a chemostat with silicate as limiting nutrient. Mar. Biol. 19: 117–126.

PARSONS, T. R. 1965. A general description of some factors governing primary production in the Strait of Georgia, Hecate Strait and Queen Charlotte Sound and the N.E. Pacific Ocean. Fish. Mar. Serv. MS Rep. 193: 34 p.

PARSONS, T. R., AND R. J. LEBRASSEUR. 1970. The availability of food to different trophic levels in the marine food chain, p. 325–343. *In* J. H. Steele [ed.] Marine food chains. Edinburgh, Oliver and Boyd.

PARSONS, T. R., R. J. LEBRASSEUR, AND W. E. BARRACLOUGH. 1970. Levels of production in the pelagic environment of the Strait of Georgia, British Columbia: a review. J. Fish. Res. Board Can. 27: 1251–1264.

PARSONS, T. R., R. L. LEBRASSEUR, J. D. FULTON, AND O. D. KENNEDY. 1969b. Production studies in the Strait of Georgia, II. Secondary production under the Fraser River plume, February to May, 1967. J. Exp. Mar. Biol. Ecol. 3: 39–50.

PARSONS, T. R., K. STEPHENS, AND R. J. LEBRASSEUR. 1969a. Production studies in the Strait of Georgia, I. Primary production under the Fraser River plume. February to May, 1967. J. Exp. Mar. Biol. Ecol. 3: 27–38.

PHIFER, L. D. 1933. Seasonal distribution and occurrence of planktonic diatoms at Friday Harbor, Washington. Univ. Wash. Publ. Oceanogr. 1: 39–81.

PHIFER, L. D., AND T. G. THOMPSON. 1937. Seasonal variations in the surface waters of San Juan Channel during the five-year period, January 1931 to December 30, 1935. J. Mar. Res. 1: 34–53.

PRITCHARD, D. W. 1967. What is an estuary: physical viewpoint, p. 3–5. *In* G. H. Lauff [ed.] Estuaries. AAAS pub. No. 83. Washington, D.C.

RILEY, G. A. 1956. Oceanography of Long Island Sound, 1952–1954. 9. Production and utilization of organic matter. Bull. Bingham Oceanogr. Collect. 15: 324–334.

RYTHER, J. H., AND W. M. DUNSTAN. 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. Science 171: 1008–1013.

RYTHER, J. H., and C. S. YENTSCH. 1958. Primary production of continental shelf waters off New York. Limnol. Oceanogr. 3: 327–335.

SEGAR, D. A., AND G. A. BERBERIAN. 1976. Oxygen depletion in the New York Bight apex: Causes and consequences. Am. Soc. Limnol. Oceanogr. Spec. Symp. 2: 220–239.

STEEMANN-NIELSEN, E. 1952. The use of radioactive carbon (^{14}C) for measuring organic production in the sea. J. Cons. 18: 117–140.

STEPHENS, K. 1968. Surface distribution of chlorophyll *a* in the Strait of Georgia, 1966 and 1967. Fish. Res. Board Can. MS Rep. 971: 26 p.

STEPHENS, K., J. D. FULTON, AND O. D. KENNEDY. 1969. Summary of biological oceanographic observations in the Strait of Georgia, 1965–1968. Fish. Res. Board Can. Tech. Rep. 110: 97 p.

STOCKNER, J. G., AND D. D. CLIFF. 1975. Marine phytoplankton production, distribution and species composition in Pendrell and Hotham Sounds, British Columbia. Fish. Mar. Serv. Tech. Rep. 516: 80 p.

1976a. Phytoplankton succession and abundance in Howe Sound, British Columbia: a coastal marine embayment under stress. Fish. Mar. Serv. Tech. Rep. 658: 24 p.

1976b. Effects of pulpmill effluent on phytoplankton production in coastal marine waters of British Columbia. J. Fish. Res. Board Can. 33: 2433–2442.

1979. Plankton ecology of Vancouver Harbor. J. Fish. Res. Board Can. 36: 1–10.

STOCKNER, J. G., D. D. CLIFF, AND D. B. BUCHANAN. 1977. Phytoplankton production and distribution in Howe Sound, British Columbia: a coastal marine embayment-fjord under stress. J. Fish. Res. Board Can. 34: 907–917.

STRICKLAND, J. D. H. 1958. Data Record and Discussion. A special B.C. coastal survey measuring chemical and light attenuation properties, June to July 1958. MS Rep. Ser. (Oceanogr. Limnol.) 30: 48 p.

1960. Measuring the production of marine phytoplankton. Bull. Fish. Res. Board Can. 122: 172 p.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1968. A practical handbook of seawater analysis. Bull. Fish. Res. Board Can. 167: 311 p.

TAKAHASHI, A., K. FUJII, AND T. R. PARSONS. 1973. Simulation study of phytoplankton photosynthesis and growth in the Fraser River estuary. Mar. Biol. 19: 102–116.

TANIGUCHI, A. 1972. Geographical variation of primary production in the western Pacific Ocean and adjacent seas with reference to the inter-relations between various parameters of primary production. Mem. Fac. Fish. Hokkaido Univ. 19(1/2): 1–34.

TULLY, J. P., AND A. J. DODIMEAD. 1957. Properties of the water in the Strait of Georgia, British Columbia, and influencing factors. J. Fish. Res. Board Can. 14: 241–319.

UTERMÖHL, H. 1958. Für vervollkommnung der quantitativen phytoplankton-methodik. Mitt. Int. Ver. Limnol. 9: 1–38.

WALDICHUK, M. 1957. Physical oceanography of the Strait of Georgia, British Columbia. J. Fish. Res. Board Can. 14: 321–486.

WALDICHUK, M., J. R. MARKERT, AND J. H. MEIKLE. 1968. Fraser River estuary, Burrard Inlet, Howe Sound and Malaspina Strait physical and chemical oceanographic data, 1957–1966. Fish Res. Board Can. MS Rep. 939: 277 p.

WINTER, D. F., K. BANSE, AND G. C. ANDERSON. 1975. The dynamics of phytoplankton blooms in Puget Sound, a fiord in the Northwestern United States. Mar. Biol. 29: 136–176.

Intertidal Catastrophes: Effect of Storms and Hurricanes on Intertidal Benthos of the Minas Basin, Bay of Fundy

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Hurricane Beulah (July 1975) and a major storm both caused catastrophic mortalities among intertidal benthos of the Minas Basin. In both events, surface sediment was scoured off the flats, resulting in high mortalities in shallow-burrowing organisms. The storm was a more severe catastrophe than the hurricane. The demonstrated high secondary productivity of these flats is difficult to reconcile with these observations; extensive lateral migration of the benthos may aid in recovery from catastrophes.

Key words: benthos, intertidal productivity, Bay of Fundy, tidal power, catastrophes, *Macoma balthica*, *Corophium volutator*

YEO, R. K., AND M. J. RISK. 1979. Intertidal catastrophes: effect of storms and hurricanes on intertidal benthos of the Minas Basin, Bay of Fundy. J. Fish. Res. Board Can. 36: 667–669.

L'ouragan Beulah (juillet 1975) et une violente tempête furent tous deux la cause de mortalités catastrophiques parmi le benthos intertidal du bassin des Mines. Dans les deux cas, le sédiment superficiel a été lavé des sèches, causant de fortes mortalités d'organismes fouisseurs de faible profondeur. La tempête fut une plus grande catastrophe que l'ouragan. Ces observations sont toutefois en contradiction avec la forte productivité de ces sèches; il se peut qu'une importante migration latérale du benthos favorise le rétablissement à la suite de catastrophes.

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THE possibility of generating power from the tides of the Bay of Fundy has been studied and discussed for many years and has recently been brought closer to reality by increases in the price of fossil fuels (Anon. 1977). Because of the possible importance of the upper reaches of the Bay of Fundy as nursery sites for commercial fisheries (Risk et al. 1977), and because of their demonstrated importance in the life cycle of many birds (Hughson 1977; Morrison 1977), it is important that as complete a picture as possible be drawn of the interaction between physical and biological processes in these regions.

Recent research has shown that the intertidal flats of the Minas Basin, which represent a large proportion of the total area of the estuary, support highly productive, low diversity assemblages of benthic invertebrates (Risk and Yeo 1977; Yeo 1978). Several species of commercially important fishes occur in the Minas Basin as larvae, juveniles, and adults, and evidence of fish predation on the intertidal invertebrates is extensive (Risk and Craig 1976; Yeo 1978). The possible effect of construction of tidal power barrages can best be evaluated when there are sufficient data, not only on the average population densities on the flats, but also on the temporal fluctuations about the averages. In this note, we report on biological and sedimentological changes caused by a hurricane and a severe storm during the summer of 1975.

Methods — Data were gathered as part of a continuing study of the intertidal flats of the Minas Basin. Sediment properties and organism densities were monitored at 12 locations, (spaced at 50-m intervals) along an intertidal transect erected from high towards low tide off Salter Head (Fig. 1). Samples were routinely collected in mid-month, from April until August.

Invertebrate populations were censused in triplicate by depressing a 1/18 m^2 plastic-core liner at least 20 cm into the sediments. This material was then sieved through 1 ϕ (0.5 mm) screens, and organisms retained were identified and counted.

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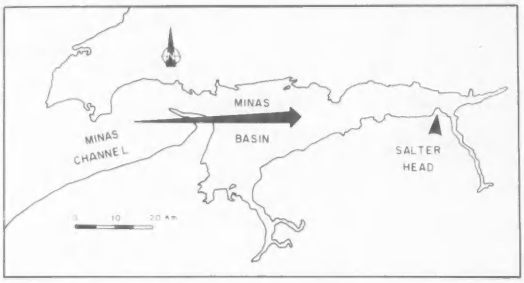

FIG. 1. Salter Head transect. Arrow shows prevailing wind directions during both the storm and the hurricane.

Grab samples of the top few centimetres of sediment were taken at the same time as invertebrate sampling. Grain size of this material was determined using standard techniques (Folk 1968). Erosion and accretion of the intertidal sediments was monitored by measuring the depth to a metal plate buried 20–30 cm below the surface.

Observations and discussion — The hurricane began the night of July 28, 1975 and blew throughout July 29. Windspeeds up to 120 km/h were recorded at Truro. Rainfall was locally heavy (up to 2 cm) and much of it was confined to a 1-h period towards low tide on July 29. The Salter Head transect was resampled on July 30. During August 19–21, 1975, the area was subjected to strong westerly winds (average velocities of about 35 km/h, gusts to 80 km/h). The wind direction of this stormy period was parallel to the maximum fetch of the Minas Basin, resulting in very strong wave activity. Storms of such intensity are not exceptional in this area — the famous Groundhog Day Storm (February 2, 1976) recorded wind velocities close to 180 km/h. The Salter Head transect was resampled on August 22.

Both the hurricane and the storm caused significant erosion of the flats. In some cases, up to 8 cm of surface material was removed (Fig. 2). Maximum erosion occurred lower in the intertidal zone (Stations 9, 10, 11) where wave activity was higher, due to the slope

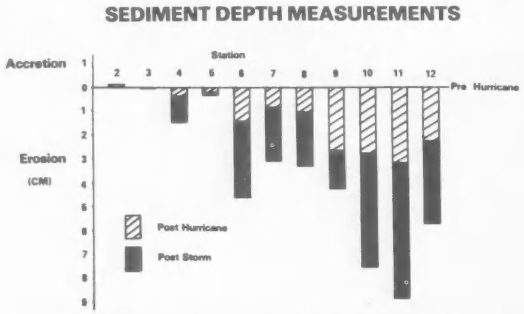

FIG. 2. Erosional changes in the level of surface sediments following storm activity (expressed relative to pre-hurricane levels).

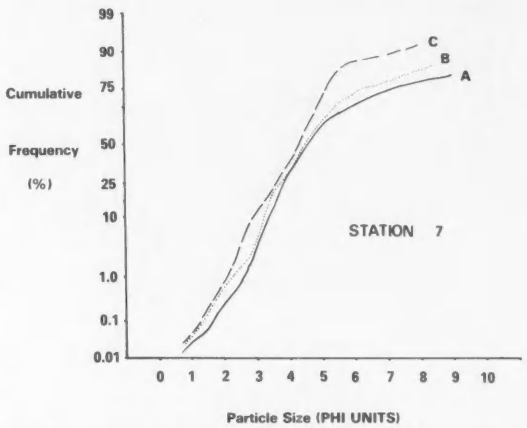

FIG. 3. Changes in grain size of surface sediments before and following storm erosion ($\phi = \log_2$ grain diameter in mm). A, prehurricane; B, posthurricane; C, poststorm.

of the flats. The result of this erosion was to leave behind a residual veneer of fine sand, significantly coarser than the material originally present on the flats (Fig. 3).

Before the hurricane, the study area supported a high-density assemblage of *Corophium volutator* and *Macoma balthica*; the hurricane caused reductions in numbers of both species (Fig. 4). The effect was most pronounced on the lower part of the flat, where population densities were highest. Reductions as a result of the storm were catastrophic; much greater than the hurricane. In the lower intertidal zone, populations were reduced to less than one tenth their original values. Continued monitoring of this coastline showed that a year after, population densities in the lower intertidal zone of the Salter Head transect were still less than one fifth original values.

Both the storm and the hurricane selectively removed the smallest *Macoma*. After the storm, only the largest *Macoma* (shell width >12 mm) remained on the flats.

Mortalities caused by abnormally low tides have already been noted in the Minas Basin (Bleakney 1972). In the case of storms and hurricanes, physical removal of organisms from the flats results in mortalities. After the storm, windrows of *Macoma*, some still alive, were found washed high up on the beach. *Macoma* still left on the flats were partly exposed, due to the extensive erosion, and were being preyed on by shorebirds. *Corophium* carcasses were observed in piles several centimetres thick, washed up into the salt marsh. The relatively greater depletion in *Corophium* numbers is due to the fact that *Corophium* is a shallow burrower, rarely going deeper than 4 cm. *Macoma*, on the other hand, may occur as deeply as 12 cm in the sediment.

Salter Head is relatively exposed, and hence susceptible to the sort of damage described in this note,

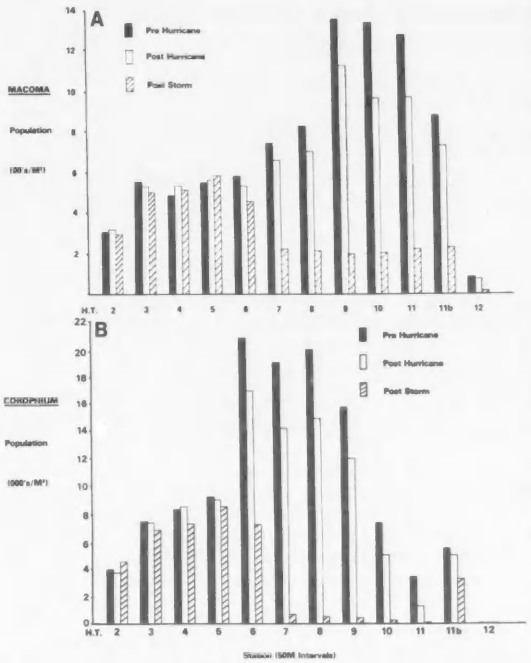

FIG. 4. Pre- and poststorm and hurricane populations of (*A*) *Macoma balthica* and (*B*) *Corophium volutator*.

but it is not unique. The same phenomena of erosion and mortality were observed at many other areas following the storm and hurricane. The Salter Head case is the most thoroughly documented, and the data are presented for illustrative purposes. How can the observation of generally high secondary productivity on these flats (Yeo 1978) be reconciled with the occurrence of catastrophic mortalities?

In general, the lateral variability in benthic population densities in the Minas Basin is not high. For example, replicate transects spaced at 25-m intervals show a percentage variability in the population density of *Macoma balthica* of 2–24%; the highest variability occurs in nearshore areas, where densities are lower and distributions patchy. Lateral migration by *Macoma* undoubtedly acts to even out the distribution of population densities. Early in spring, just after the ice has left the flats, individual *Macoma* may be observed migrating laterally at the surface. Whatever the reasons for such behavior, the result is to repopulate ice-scoured areas. Lateral migrations occur throughout the summer, but are not as common as in early spring. (This migratory behavior in *Macoma* has been attributed to trematode infection: several of the migrating *Macoma* in the Minas Basin were sampled, and found free of trematodes.) Square-metre plots from which all *Macoma* were removed early in summer are repopulated by the end of summer (to ~75% of original densities) by lateral migration of adult *Macoma* from adjacent areas. *Corophium* populations are also able to invade vacant areas of suitable substrate; in this case, the process is accelerated by the swimming and foraging activities of the adults.

Areas hardest hit by the storm and hurricane events of 1975 still had not recovered initial population densities by 1977 (R. Hirtle, Department of Geology, McMaster University, Hamilton, Ont. L8S 4M1, personal communication). On the other hand, areas that are scoured free of *Macoma* by ice often exhibit, by midsummer, population densities of *Macoma* indistinguishable from and homogeneous with adjacent areas. Evidently, there is a spectrum of catastrophes. The redistributive activities of the benthos can produce recovery from a minor catastrophe, such as an ice scour, in a few months. Recovery from major catastrophes may take a decade or more. Any baseline studies of this ecosystem designed to assess its importance to fisheries should include an evaluation of the degree to which the normal is overprinted by the exceptional.

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ANON. 1977. Reassessment of Fundy Tidal Power. Bay of Fundy Tidal Power Review Board, Ottawa, Ont. 57 p.

BLEAKNEY, J. S. 1972. Ecological implications of annual variation in tidal extremes. Ecology 53: 933–938.

HUGHSON, W. B. 1977. Fundy avifauna: an overview. Fundy Tidal Power and the Environment, Acadia Univ. Inst. 28: 180–186.

FOLK, R. L. 1968. Petrology of sedimentary rocks. Hemphill's Book Store, Austin, Tex. 170 p.

MORRISON, R. I. 1977. Use of the Bay of Fundy by shorebirds. Fundy Tidal Power and the Environment, Acadia Univ. Inst. 28: 187–199.

RISK, M. J., AND H. D. CRAIG. 1976. Flatfish feeding traces in the Minas Basin. Sediment. Petrol. 46: 411–413.

RISK, M. J., AND R. K. YEO. 1977. Possible effects of tidal power: aspects of the trophic structure of the Minas Basin, Bay of Fundy. Am. Zool. 17: 907.

RISK, M. J., R. K. YEO, AND H. D. CRAIG. 1977. Aspects of marine ecology of the Minas Basin relevant to tidal power development. Fundy Tidal Power and the Environment, Acadia Univ. Inst. 28: 164–179.

YEO, R. K. 1978. Animal–sediment relationships and the ecology of the intertidal mudflat environment, Minas Basin, Bay of Fundy, Nova Scotia. M.Sc. thesis, McMaster University, Hamilton, Ont. 396 p.

Decrease in Mercury Content of Fishes in Ball Lake, Ontario, Since Imposition of Controls on Mercury Discharges

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ARMSTRONG, F. A. J., AND D. P. SCOTT. 1979. Decrease in mercury content of fishes in Ball Lake, Ontario, since imposition of controls on mercury discharges. J. Fish. Res. Board Can. 36: 670–672.

Weight, fork length, and white muscle mercury concentrations of walleye (*Stizostedion vitreum vitreum*), northern pike (*Esox lucius*), and lake whitefish (*Coregonus clupeaformis*) from Ball Lake, northwestern Ontario, were compared for 1971, 1972, and 1976. The lake had received mercury from 1962 to 1970; since then there have been increasingly rigorous controls. Adjusted mean white muscle mercury concentrations for walleye at the group mean length of 444 mm were 1.99, 2.71, and 1.39 mg/kg, respectively, for the three sample years. Corresponding values for pike 594 mm long were 5.05, 5.72, and 1.80 mg/kg. Whitefish values for 1972 and 1976 were 0.62 and 0.42 at 441 mm fork length. The changes are discussed in relation to the decreased input of mercury to the lake and it is suggested that mercury in suspension may be a controlling factor.

Key words: mercury contamination, walleye, pike, whitefish, Ball Lake

ARMSTRONG, F. A. J., AND D. P. SCOTT. 1979. Decrease in mercury content of fishes in Ball Lake, Ontario, since imposition of controls on mercury discharges. J. Fish. Res. Board Can. 36: 670–672.

Nous comparons, pour les années 1971, 1972 et 1976, le poids, la longueur à la fourche et les teneurs en mercure du muscle blanc du doré jaune (*Stizostedion vitreum vitreum*), du grand brochet (*Esox lucius*) et du grand corégone (*Coregonus clupeaformis*) du lac Ball, dans le nord-ouest de l'Ontario. On avait déversé du mercure dans ce lac de 1962 à 1970; depuis, des contrôles de plus en plus sévères ont été établis. Les concentrations moyennes ajustées de mercure dans le muscle blanc du doré jaune à la longueur moyenne du groupe de 444 mm sont 1,99, 2,71 et 1,39 mg/kg respectivement pour les trois années d'échantillonnage. Les valeurs correspondantes pour le grand brochet de 594 mm de longueur sont de 5,05, 5,72 et 1,80 mg/kg. Chez le grand corégone, les valeurs de 1972 et 1976 sont de 0,62 et 0,42 à 441 mm de longueur à la fourche. Nous analysons ces changements en relation avec la diminution des apports de mercure dans le lac et suggérons que le mercure en suspension peut être un facteur de contrôle.

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BALL Lake, in the Wabigoon–English–Winnipeg River system of northwestern Ontario, contains fish with high white muscle mercury concentrations (Bligh 1970; Scott and Armstrong 1972; Annett et al. 1975; Bishop and Neary 1976). Native people in the area eat fish from this and other polluted lakes and are known to have raised blood-mercury levels (Anon. 1973).

The lake (50°17′N, 94°00′W) is the next downstream from the heavily polluted Clay Lake and was contaminated by the same source. Mercury discharges from a chlor-alkali plant in Dryden, Ontario, were uncontrolled from 1962 to 1970, during which time some 10 000 kg of the metal were discharged into the Wabigoon River (Armstrong and Hamilton 1973). From 1970 until the end of 1975, discharges were controlled and were reported to be 3% of what they had been. In October 1975, the plant was converted to a process that does not use mercury and discharges should have ceased altogether.

We have data for white muscle mercury concentrations of walleye (*Stizostedion vitreum vitreum*) and northern pike (*Esox lucius*), with weights and fork lengths, for 1971 for Ball Lake. Annett et al. (1975) give means of mercury concentrations for 1972 samples of these fishes and some others, including lake whitefish (*Coregonus clupeaformis*). These authors generously made available to us their individual analyses and measurements. This made it possible, with the addition of data from a further collection in 1976 of the three fishes named above, to compare mercury concentrations in white muscle for 1971, 1972 and 1976 and to look for any changes.

Materials and methods — The collections of 1971 and 1976, by Freshwater Institute staff were made with stand-

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TABLE 1. Within-sample regressions, log [Hg] on log fork length, and log weight on log fork length, with adjusted group means. B_0 = intercept; B_1 = slope; r^2 = coefficient of determination. Significance by superscript: [0] is $P < 0.9$; [1] is $0.9 \leq P < 0.99$; [2] is $0.99 \leq P < 0.999$; [3] is $0.999 \leq P$.

Species	Year	n	log [Hg] on log length			log weight on log length			Adjusted group means[a]			
			$\hat{B}_0$	$\hat{B}_1$	r^2	$\hat{B}_0$	$\hat{B}_1$	r^2	Weight (g)	Length (mm)	Hg (mg/kg)	Burden (μg)
Walleye	71	60	−1.372	1.014[1]	0.046	−0.962	3.026[3]	0.944	1054	444	1.99	2097
	72	45	0.431	0.001[0]	1×10^{-7}	−2.126	3.069[3]	0.862	849	444	2.71	2301
	76	46	−1.534	1.018[3]	0.366	−2.029	3.048[3]	0.993	979	444	1.39	1361
Pike	71	20	−3.404	2.315[1]	0.186	−1.688	2.746[3]	0.693	1520	594	5.05	7676
	72	15	−0.082	0.474[0]	0.008	−3.119	3.481[3]	0.953	1136	594	5.72	6498
	76	46	−3.792	2.281[3]	0.324	−1.792	2.770[3]	0.920	1323	594	1.80	2381
Whitefish	72	15	−3.284	1.871[0]	0.020	−0.918	2.388[3]	0.694	1023	441	0.62	634
	76	45	−3.397	1.838[1]	0.120	−1.835	2.997[3]	0.937	1241	441	0.42	521

[a]Geometric means from retransformed log data.

ard ganged gillnets to capture fish with as wide a size range as possible. Fork lengths and weights were individually recorded and the dorsal white muscle analyzed for mercury content by the method of Armstrong and Uthe (1971). The 1972 collection was made by angling and gillnetting (Annett et al. 1975); analyses were by a similar method. In all collections the mercury concentrations were reported as means of duplicate determinations, i.e. two readings from separate portions of digested white muscle. In spite of our efforts, some of the sample length (and therefore weight) ranges are shorter than is desirable when related to the variance in mercury concentrations; also, some sample numbers are small related to sample variances.

Statistical analyses have been made (Dixon and Massey 1957) first for differences in mercury concentrations in white muscle related to size of fish between years within species, then for differences between condition factors of the fish between years. Condition factor was measured simply as differences in weight–length regressions between years. Standard analyses of covariance (Dixon and Massey 1957) were used to test the significance of differences in slopes and adjusted group means of the bivariate regressions of log mercury concentration on log length, and log weight on log length within species between years.

Results and discussion — Table 1 shows the coefficients of the linear regressions, and the associated adjusted group means of the data collections. Variance ratios (F) for the analysis of covariance tests for adjusted group means and for slopes of the regressions are shown in Table 2. There are highly significant differences in adjusted group mean weights at the group mean lengths of all three species in the three sampling years. There are some differences in the slopes of the log weight on log length relationships among pike samples. Differences in mean mercury concentration at group mean length are highly significant for walleye and pike, with 1972 highest and 1976 least. In spite of the lack of significance between whitefish samples, it should be noted that the ranking of years within species for both relationships is the same throughout, i.e. for weight 1971 > 1976 > 1972, and for mercury concentration 1972 > 1971 > 1976, as shown in Table 1.

At least part of these differences are probably due to decrease of mercury pollution of the lake. The matter is not straightforward, however. In the first place, there was a moderate *increase* in mercury in walleye and pike from 1971 to 1972, in spite of a reasonable expectation that pollution of the lake should have been decreasing. We do not believe the increase to be an artifact of sampling, since the pattern is consistent in both species. Both species showed a concomitant *decrease* in adjusted mean weight; it is tempting to speculate on a cause-and-effect relationship between the mercury concentration rise and

TABLE 2. Analyses of covariance, log [Hg] on log fork length, and log weight on log fork length, *between* years, *within* species. Significances by superscript as in Table 1.

Species	Log [Hg] on log length					Log weight on log length				
	Means		Year order (decreasing)	Slopes		Means		Year order (decreasing)	Slopes	
	F	df		F	df	F	df		F	df
Walleye	12.786	(2; 147)[3]	72; 71; 76	0.734	(2; 145)[0]	55.064	(2; 147)[3]	71; 76; 72	0.032	(2; 145)[0]
Pike	24.857	(2; 77)[3]	72; 71; 76	1.220	(2; 75)[0]	8.282	(2; 77)[3]	71; 76; 72	2.998	(2; 75)[1]
Whitefish	2.303	(1; 57)[0]	72; 76	0.000	(1; 56)[0]	58.532	(1; 57)[3]	76; 72	1.681	(1; 56)[1]

the weight decrease. Since turnover rates of mercury in fish are known to be very slow (Lockhart et al. 1972), some of the increase in mercury concentrations might conceivably be accounted for simply by the weight loss (for walleye, 1.99 mg/kg × 1054/849 = 2.47 mg/kg [actual = 2.71]; for pike, 5.05 mg/kg × 1520/1136 = 6.76 mg/kg [actual = 5.72]). Mercury burden of these fishes changed relatively little between 1971 and 1972, but decreased substantially between 1972 and 1976.

The much greater *decrease* in mercury concentration in walleye and pike between 1972 and 1976, coupled with moderate weight increases, can probably be partly accounted for in the same manner but the proportion is much less than half of the observed decrease for walleye (2.71 × 849/979 = 2.35 [actual = 1.39]) and pike (5.72 × 1136/1323 = 4.91 [actual = 1.80]), and about half for whitefish (0.62 × 1023/1241 = 0.51 [actual = 0.42]). It appears to us that the remaining decrease can be attributed to decreased mercury pollution of the lake.

One must ask how a decrease in mercury input to the lake, attributable to pollution control, effects such decreases in mercury contamination of the fish. It has been supposed that the mercury taken up by fish is methylmercury generated from inorganic mercury in the sediments by microorganisms and mobilized because of its greater solubility (Jensen and Jernelöv 1969). If this were the *whole* explanation, then a decrease in mercury input should not greatly affect the methylation process and uptake of methylmercury. Over the time period in question, we do not expect there to have been much decrease in sediment mercury levels (and thus the amount mobilized by methylation), but there should have been a very large decrease in water column mercury content coming directly from the contamination source. We suggest that this decrease may be responsible for much of the decreased mercury content of the fish. We have no direct observations of mercury in suspension in this system during the years of heavy pollution (before 1970), but Armstrong and Hamilton (1973) estimated that in Clay Lake the mercury content of suspended matter may have been about 30 mg/kg, compared with a measured 3–4 mg/kg in 1971 after controls were imposed. We have no such data for Ball Lake, but it is reasonable to suppose that levels may have been similar due to the lakes' proximity in the watershed.

We suggest, also, that if mercury levels in fish are indeed controlled, in part, by mercury in suspension, it follows that methylation should occur in the water column since the element in fish is present as methylmercury. Methylation in the fish themselves was looked for by Pennachioni et al. (1976) but was not found.

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ANNETT, C. S., F. M. D'ITRI, J. R. FORD, AND H. H. PRINCE. 1975. Mercury in fish and water fowl from Ball Lake, Ontario. J. Environ. Qual. 4: 219–222.

ANON. 1973. Final report. Task force on organic mercury in the environment. Grassy Narrows and White Dog, Ontario. Health and Welfare Canada. 20 + 5 + 20 p.

ARMSTRONG, F. A. J., AND A. L. HAMILTON. 1973. Pathways of mercury in a polluted Northwestern Ontario lake, p. 131–155. *In* P. E. Singer [ed.] Trace metals and metal organic interactions in natural waters. Ann Arbor Science Publications, Ann Arbor, Mich.

ARMSTRONG, F. A. J., AND J. F. UTHE. 1971. Semi-automated determination of mercury in animal tissues. Atomic Absorpt. Newsl. 10: 101–103.

BISHOP, J. N., AND B. P. NEARY. 1976. Mercury levels in fish from northwestern Ontario, 1970–1975. Ontario Min. of Environ. 84 p.

BLIGH, E. G. 1970. Mercury and the contamination of freshwater fish. Fish. Res. Board Can. MS Rep. 1088: 22 p.

DIXON, W. J., AND F. J. MASSEY JR. 1957. Introduction to statistical analysis. 2nd ed. McGraw-Hill Book Company Inc., New York, N.Y. xiii + 488 p.

JENSEN, S., AND A. JERNELÖV. 1969. Biological methylation of mercury in aquatic organisms. Nature (Lond.) 223: 753–754.

LOCKHART, W. L., J. F. UTHE, A. R. KENNEY, AND P. M. MEHRLE. 1972. Methylmercury in northern pike (*Esox lucius*): distribution, elimination, and some biochemical characteristics of contaminated fish. J. Fish. Res. Board Can. 29: 1519–1523.

PENNACHIONI, A., R. MARCHETTI, AND G. E. GAGGINO. 1976. Inability of fish to methylate mercuric chloride *in vivo*. J. Environ. Qual. 5: 451–454.

SCOTT, D. P., AND F. A. J. ARMSTRONG. 1972. Mercury concentration in relation to size in several species of freshwater fishes from Manitoba and northwestern Ontario. J. Fish. Res. Board Can. 29: 1685–1690.

Role of Tubificid Worms on Nitrogen Transformations in Stream Sediment

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Sediments obtained from Swifts Brook were highly organic whereas those from Canagagigue Creek were coarse-textured with relatively little organic content. Columns of sediment were overlain with continuously aerated 10 mg/L nitrate-N solution or distilled water. When columns of Canagagigue sediment with tubificid worms (*Tubifex tubifex* and *Limnodrilus hoffmeisteri*) were incubated for 33 d they lost nitrate-N at a significantly higher rate ($P < 0.01$) than did columns without worms. Following the initial period of nitrate disappearance (resulting from denitrification in the sediment) nitrate-N increased again, this increase being greatest in the presence of worms. The increasing concentration of nitrate-N probably resulted from nitrification which was masked early in the experiment by rapid denitrification. In columns with Swifts Brook sediment, added nitrate disappeared rapidly but no significant difference was observed between the columns with worms and those without; nitrate-N concentration was never observed to increase. Possibly both nitrification and denitrification are also enhanced here by the presence of tubificids but the effects are masked.

Key words: tubificids, denitrification, nitrification, stream sediment, nitrate, ammonium, fecal pellets

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Les sédiments provenant du ruisseau Swifts sont fortement organiques, alors que ceux du ruisseau Canagagique ont une texture grossière et contiennent relativement peu de matière organique. Les colonnes de sédiment ont été recouvertes d'une solution de 10 mg/L de nitrate-N ou d'eau distillée aérées de façon continue. Après incubation de 33 d, les colonnes de sédiment de Canagagique contenant des vers tubificides (*Tubifex tubifex* et *Limnodrilus hoffmeisteri*) perdent le nitrate-N à une vitesse sensiblement plus grande ($P < 0{,}01$) que les colonnes dépourvues de vers. Après une période initiale de disparition du nitrate (résultant de la dénitrification dans le sédiment), le nitrate-N augmente de nouveau, cette augmentation étant plus prononcée en présence de vers. La concentration croissante de nitrate-N résulte probablement d'une nitrification qui est masquée au début de l'expérience par une rapide dénitrification. Dans les colonnes de sédiment du ruisseau Swifts, le nitrate ajouté disparaît rapidement, mais il n'y a pas de différence significative entre les colonnes contenant des vers et celles qui en sont dépourvues; on n'observe aucune augmentation de concentration du nitrate-N. Il est possible que la nitrification aussi bien que la dénitrification soient accentuées, ici aussi, par la présence de tubificides, mais les effets sont masqués.

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IN an earlier study (Kaushik and Robinson 1976) of a spring-fed stream, Swifts Brook, it was observed that as the water flowed downstream its nitrate-N concentration decreased from an average value of 5.53 mg/L at the spring to 2.40 mg/L at the 2 km downstream station. Other studies on the stream (Kaushik et al. 1975) ruled out dilution as a probable factor for this decrease in nitrate concentration and indicated that uptake by macrophytes and microbial immobilization could only account for <10% of the observed loss of N from the water. Such losses of N have been reported from lake ecosystems (Keeney 1973) and denitrification in the anoxic zone (sediment and water) has been ascertained as the main causal factor (Chen et al. 1972). It was presumed, therefore, that in Swifts Brook the observed loss was also due to denitrification. However, it seemed likely that the process could occur only in the sediment as the water in the stream was always well oxygenated. When columns of stream sediment were reconstituted in plexiglass tubes and overlain with constantly aerated water containing ~10 mg/L nitrate-N, a rapid loss of nitrogen from the water resulted (Sain et al. 1977). Subsequently Chatarpaul and Robinson (1979) using $^{15}NO_3$-N, conclusively showed that this loss was because of denitrification. To determine whether this phe-

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nomenon of denitrification also occurred in the sediment from other streams, column experiments were performed with sediments from Canagagigue Creek, near Elmira, Ontario. During the course of preliminary experiments it was observed that certain columns always lost nitrate much more rapidly than did other replicates of the same treatment. Closer examination of the columns revealed that species of oligochaete, worms, *Limnodrilus hoffmeisteri*, *Tubifex tubifex*, and perhaps others, were usually present in large numbers in these seemingly anomalous columns while few or no worms were present in columns that lost nitrate-N more slowly. In the present paper, we describe the results of experiments in which we studied the apparent association of oligochaete worms in stream sediment with a high rate of nitrate loss from overlying water.

Materials and methods — Sediment samples used in this study were taken either from the East branch of Canagagigue Creek, a perennial, well-defined stream that passes through areas of developed farmland interspersed with deciduous and coniferous trees or from Swifts Brook, described by Kaushik and Robinson (1976). Sediment was collected by using a 60 cm long plexiglass column of 6.40 cm OD and 0.40 cm wall thickness. One end of the column was sharpened to facilitate penetration. In the field, the sharpened end of the column was pushed ~10 cm into the sediment, its top end closed with a rubber stopper and the column pulled out with the intact core of sediment. Sediment collected in this manner was thoroughly mixed in a plastic bucket. This eliminated likely variation in the physicochemical properties of the sediment from different columns. Oligochaete worms from the sediment were removed by using a Pasteur pipet. The worm-free sediment was then transferred to plexiglass columns (60 cm × 6.40 cm OD, 5.6 cm ID) to a depth of 10 cm. Air bubbles were purged by a light tapping of the columns and adhering sediment was washed from the sides of the column walls with deionized water. After the sediment columns had settled overnight at 2°C, the supernatant water was siphoned off and the sediment overlain with either 500 mL of KNO_3 (10 mg/L) or with distilled water. A gas mixture of 21% oxygen and 79% helium was introduced by a sparger located about 1 cm above the sediment–water interface. This procedure was intended to create a little turbulence and to keep the supernatant solution aerated in an effort to simulate stream conditions. Triplicate columns for each treatment were incubated in the dark for 33 d either at room temperature (22°C) or at 15°C in a controlled environmental chamber. Nitrate-N concentration in the supernatant was monitored routinely by removing 2.5-mL samples and analyzing for NO_3-N using a modified chromotropic acid method (West and Ramachandran 1966). Samples of sediment from the two streams were dried at 70°C, ground, and passed through a 30-mesh screen (595 nm). Triplicate samples of each type of sediment were then analyzed for ammonium-N, organic-N, nitrate-N, and combustible matter. Methods used for these analyses are listed in Sain et al. (1977).

The worms *Limnodrilus hoffmeisteri* and *Tubifex tubifex* used in some columns were obtained from Toronto Harbour near Hanlan's Point. The worms were taken in the sediment with an Ekman grab and sieved in the field in a wash bucket of 0.5-mm pore size. The residues were placed in large plastic pails with lake water and stored at 10°C in a controlled environmental chamber.

The data obtained were subjected to polynomial regression and correlation analyses with the aid of a computer (Tektronic 4051). Values for various constants were calculated and the regression lines of the fitted equation drawn in Fig. 1–3. Two-way analysis of variance tests were performed on the data (Steel and Torrie 1960).

Results — Characteristics of the sediment taken from Canagagigue Creek and Swifts Brook are shown in Table 1. Sediment from Swifts Brook was collected from a site about 200 m downstream of the spring where the stream passed through a cedar stand. Swifts Brook has all the characteristics of a headwater and its sediment had a much higher percentage of organic matter than did that from Canagagigue Creek.

The first experiment involved six columns containing 10 cm sediment from Canagagigue Creek and 500 mL of 10 mg/L KNO_3 solution. Three of these each received 40 oligochaete worms (a mixture of *L. hoffmeisteri* and *T. tubifex*). The experiment was run at room temperature (22°C) for 33 d and results are shown in Fig. 1. The columns without worms lost nitrate-N gradually to about 2 mg/L at the end of incubation. In contrast, columns with worms lost nitrate-N at a much faster rate, reaching 1 mg/L level in just 12 d. However, in the latter case, after this initial decrease, the nitrate-N concentration increased to about 2 mg/L in the next 4 d and then declined to about 0.5 mg/L.

These results indicated that not only did the presence

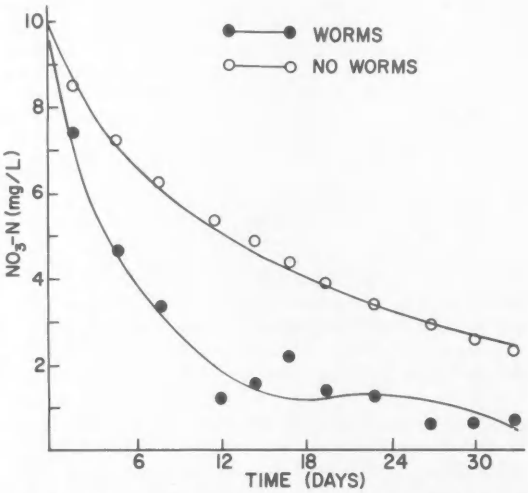

FIG. 1. Changes in the nitrate-N concentration in the aerated nitrate solution overlying Canagagigue sediment with and without oligochaete worms incubated at room temperature for 33 d. Mean of three replicates with third order regression line fitted.

TABLE 1. Some characteristics of the sediments taken from Canagagigue Creek and Swifts Brook. Mean ± SD (air-dry basis).

	Canagagigue Creek	Swifts Brook
pH	7.2	7.3
NH_4-N (μg/g)	15.0±1.0	15.01±0.75
NO_3-N (μg/g)	0	Traces
Org. N (%)	0.3±0.02	1.58±0.03
Combustible matter (%)	9.60±0.02	60.00±0.25
Carbon:nitrogen ratio	16:1	19:1

of worms in the columns change the rate of loss of nitrate-N originally added to the supernatant but also appeared to change the production of nitrate-N. A slightly more elaborate experiment was set up to confirm these findings.

Six sets of triplicate columns with Canagagigue and Swifts Brook sediment were prepared as follows:

Column no.	Sediment type	Supernatant solution	Worms
1, 2, 3	Canagagigue	500 mL KNO_3 (10 mg/L)	40
4, 5, 6	Canagagigue	500 mL KNO_3 (10 mg/L)	—
7, 8, 9	Swifts Brook	500 mL KNO_3 (10 mg/L)	40
10, 11, 12	Swifts Brook	500 mL KNO_3 (10 mg/L)	—
13, 14, 15	Swifts Brook	500 mL H_2O	40
16, 17, 18	Swifts Brook	500 mL H_2O	—

All the columns were kept for 33 d at 15°C in a controlled environmental chamber and the nitrate-N concentration in the continuously aerated supernatant was monitored at various time intervals.

In the columns with Swifts Brook sediment and distilled water, nitrate was not detected in the supernatant in either the presence or absence of worms. Columns with Swifts Brook sediment overlain with nitrate-N solution showed a rapid decrease in nitrate concentration from an initial value of 10 mg/L to ~2 mg/L in 12 d (Fig. 2). However, the presence of worms did not alter the rate of nitrate loss from supernatant in contrast to the result obtained with Canagagigue sediment. Moreover, in the Canagagigue columns containing worms, the rapid decrease in the first 12 d was followed by a significant increase in nitrate-N concentration (Fig. 2).

A third experiment was designed to measure nitrate production under these experimental conditions. Canagagigue Creek sediment was placed in two sets of triplicate columns and was overlain with continuously aerated distilled water. One set of columns received 40 oligochaete worms each. All the columns were kept for 33 d at 15°C in a controlled environmental chamber and the nitrate-N concentration was monitored. Columns without worms showed a gradual increase in nitrate-N concentration to ~2 mg/L in 12 d and this concentration was maintained until about the end of the experiment (Fig. 3). In columns with worms there

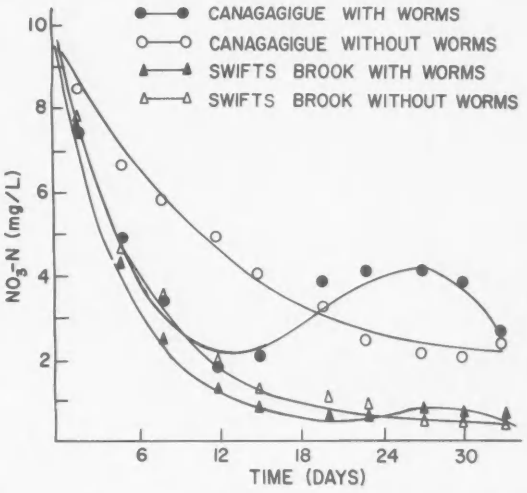

FIG. 2. Changes in the nitrate-N concentration in the aerated nitrate solution overlying Canagagigue and Swifts Brook sediments with and without oligochaete worms incubated at 15°C for 33 d. Mean of three replicates with third order regression line fitted.

was only a slight change in NO_3-N until day 12. Then the concentration rapidly increased to ~7 mg/L and maintained approximately that level until the end of the experiment.

Regression analysis of the data for the columns with worms (Fig. 1–3) showed that they follow a third order function of the form $N_c = a + bt + ct^2 + dt^3$ where N_c = nitrate concentration (mg) and t = time (days), while a, b, c, and d are constants for each curve. The values of the coefficients a, b, c, and d for equations fitted to all of the data (Fig. 1–3) are given in Table 2. Regression analysis of the data for the columns without worms could follow a second order function of the form $N_c = a + bt + ct^2$. However, to maintain uniformity a third order function has been applied to these data also and are shown in Table 2. The coefficient of determination (r^2) for the equations ranged between 0.86 and 0.98 (Table 2). Two-way analysis of variance tests performed on the data showed that the presence of the worms caused significant ($P < 0.01$) differences in both nitrate removal and nitrate production rates in Canagagigue sediment.

Discussion — Most studies that have been made on nitrogen transformations in water sediment systems pertain to flooded soils or lake ecosystems (Keeney 1973; Patrick et al. 1976). Such studies have established the possibility of occurrence of denitrification in lake sediments and flooded soils. That denitrification could also occur in sediments in streams, even though streams normally have well-oxygenated water, has been emphasized only recently (Kaushik et al. 1975; Toms et

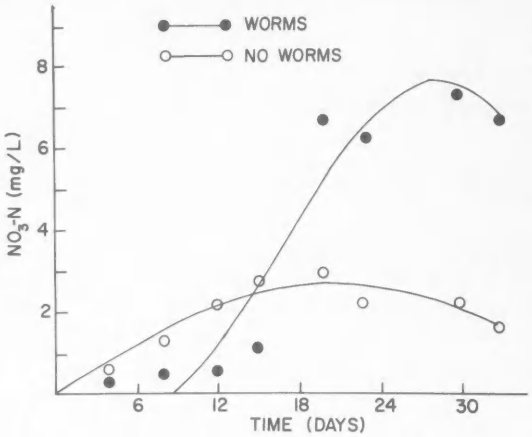

FIG. 3. Density of *M. relicta* taken in vertical net tows distilled water overlying Canagagigue sediment with and without oligochaete worms incubated at 15°C for 33 d. Mean of three replicates with third order regression line fitted.

al. 1975; Robinson et al. 1979) and only a few experimental and field studies have been reported to support this contention. Results from our experiments with both Canagagigue Creek and Swifts Brook sediment confirmed our earlier findings (Sain et al. 1977) that nitrogen disappears rapidly from well-oxygenated nitrate-containing water overlying columns of stream sediment. Such losses of nitrate-N from water–sediment systems have also been reported by Toms et al. (1975) and Van Kessel (1977). These authors, along with Sain et al. (1977), have reported the effects of such factors as pH, temperature, oxygen, and nitrate concentration of the overlying water and thickness of the stream sediment layer on the rate of removal of nitrate by denitrification. However, the role of benthic organisms in this regard has not been fully elucidated.

Experiments with Canagagigue sediment show clearly that oligochaete worms significantly increased the rate of denitrification as well as that of nitrate production. We are not aware of any study on the role of oligochaete worms in nitrogen transformations in water–sediment systems except for a paper by Kikuchi and Kurihara (1977) that appeared while the present study was in progress. To study the effects of tubificids, Kikuchi and Kurihara (1977) placed 200 g of rice field soil in vials of 6 cm diameter and topped the soil with 2 cm of water. Some vials received 50 tubificids (*L. socialis*, considered by some as a junior synonym of *L. hoffmeisteri*, and *Branchiura sowerbyi*) each. During the 75-d incubation at 25°C they noticed that until ~45 d the vials with worms had much less nitrate + nitrite in the overlying water than did those without worms. After 45 d, however, the nitrate + nitrite concentrations in the vials with worms increased rapidly. These results are thus similar to those obtained in the present study (Fig. 3) and Kikuchi and Kurihara (1977) concluded that the tubificids inhibited nitrification in the earlier stages of incubation. We believe that the lower levels of nitrate in the initial stages resulted from rapid denitrification in the vials with worms.

It is likely that diffusion and mechanical transfer of both nitrate and oxygen to the deeper zones of sediment are enhanced by the burrowing activity of the oligochaete worms. This is evident from the work of Edwards (1958) and Davis (1974). Edwards (1958) showed that introduction of larvae of *Chironomus riparius* in settled activated sludge resulted in an increase in depth of the oxidized zone of the sediment. Similarly, Davis (1974) reported that tubificid worms increased the redox potential of lake sediments at 1–4 cm depth and the oxidized layer was deepened. However, this deepening of the oxidized zone is not likely to be uniform and some microsites with reducing conditions are likely to occur. The increased diffusion and transfer of nitrate to such microsites might thus increase the rate of denitrification as noted in the present study

TABLE 2. Values of the coefficients a, b, c, and d of equations with the general form $N_c = a + bt + ct^2 + dt^3$ fitted to data (Fig. 1–3) for NO_3-N changes in water above the sediment. Mean values of three replicates ± SE (rounding of coefficients may yield values slightly different from those shown in Fig. 1–3).

Figures	Treatment	Coefficients				
		a	b	c	d	r^2
Fig. 1	No worms	9.581±0.121	−0.513±0.035	0.014±0.003	0.00015±0.00005	0.99
	Worms	9.586±0.277	−1.193±0.080	0.054±0.006	−0.00081±0.00012	0.97
Fig. 2[a]	No worms	9.757±0.147	−1.126±0.045	0.046±0.003	−0.00061±0.00006	0.99
	Worms	9.790±0.168	−1.304±0.050	0.058±0.004	−0.00083±0.00007	0.99
Fig. 2[b]	No worms	9.697±0.200	−0.587±0.059	0.015±0.004	−0.00010±0.00009	0.98
	Worms	10.100±0.180	−1.503±0.054	0.088±0.004	−0.00150±0.00008	0.98
Fig. 3	No worms	−0.182±0.180	0.261±0.059	−0.005±0.004	−0.00003±0.00009	0.86
	Worms	0.558±0.561	−0.508±0.160	0.061±0.012	−0.00122±0.00023	0.91

[a]Swifts Brook sediment.
[b]Canagagigue sediment.

(Fig. 2). It is interesting that on addition of *C. riparius* larvae to sludge, Edwards (1958) not only recorded an increase in redox potential of the surface sediment but also an increase in the concentration of ammonia and a decrease in the concentration of nitrate-N in water overlying sludge. Recently Anderson (1976) reported that when he added the larvae of *C. plumosus* to lake sediment, he observed an increase in denitrification of nitrate added to the overlying water.

The tubificid worms enhanced denitrification in Canagagigue sediment initially and then stimulated nitrate production (Fig. 2). Oligochaete worms are known to feed selectively (Brinkhurst et al. 1972) and produce fecal pellets that contain a higher percentage of organic matter than is contained in the sediment on which they feed. Since these fecal pellets are deposited on the surface, their decomposition can lead to production of larger quantities of nitrate than is produced as a result of decomposition in the sediment. Also, production of ammonia by the worms and its subsequent conversion to nitrate could contribute to increased nitrate production in columns with oligochaete worms.

Oligochaete worms had little if any effect on the apparent rate of nitrate loss from Swifts Brook sediment (Fig. 2). One can only speculate on the reasons at this time. One possibility is that the rates of both nitrification and denitrification in Swifts Brook sediment are enhanced by the presence of worms but the net result is unchanged.

The two sediments are, of course, very different in their composition (Table 1), that from Canagagigue having very much less organic matter than that from Swifts Brook. One could therefore expect differences in the biological activity in the two sediments as well as differences in their physical properties. Thus it seems reasonable that quite different factors might limit denitrification in the two sediments, particularly since a number of variables such as diffusion rate, mixing by gas production and carbon availability are known to affect the process rate (Robinson 1977). Whichever of these factors limits denitrification in the Swifts Brook sediment, it may be relatively unaffected by the presence of worms.

A further difference in the two sediments is shown by the fact that nitrate-N was never observed to increase in water overlying Swifts Brook sediment although this was always observed with Canagagigue sediment and was influenced by the presence of worms. We cannot conclude that nitrification does not go on in or at the surface of Swifts Brook sediment. However, if it does, the rate of denitrification is rapid enough to prevent the increase in nitrate from reaching detectable levels. It seems probable that the rate of nitrate production in Swifts Brook sediment is less than in that of Canagagigue because as pointed out by Painter (1977), the $K_m(O_2)$ for heterotrophs is generally lower than that for nitrifiers. We would expect that in sediment rich in carbon, heterotrophs will compete successfully for scarce oxygen conveyed into the sediment by diffusion or by mixing.

The fact that tubificids influence both nitrification and denitrification rates makes it difficult to quantify their effect without the use of tracers such as ^{15}N. Nevertheless, the qualitative evidence presented here suggests that the tubificids, indicators of pollution, may also be agents of environmental improvement.

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ANDERSON, J. M. 1976. Importance of the denitrification process for the rate of degradation of organic matter in lake sediments, p. 357–362. *In* H. L. Golterman [ed.] Interactions between sediments and freshwater. Junk Publishing Co., Amsterdam.

BRINKHURST, R. O., K. E. CHUA, AND N. K. KAUSHIK. 1972. Interspecific interactions and selective feeding by Tubificid Oligochaetes. Limnol. Oceanogr. 17: 122–133.

CHATARPAUL, L., AND J. B. ROBINSON. 1979. Nitrogen transformations in stream sediments: ^{15}N studies. *In* Proc. ASTM D-19 Symp. Fort Lauderdale, Fla. (In press)

CHEN, R. L., D. R. KEENEY, D. A. GRAETZ, AND A. J. HOLDING. 1972. Denitrification and nitrate reduction in Wisconsin lake sediments. J. Environ. Qual. 1: 158–162.

DAVIS, R. B. 1974. Tubificids alter profiles of redox potential and pH in profundal lake sediment. Limnol. Oceanogr. 19: 342–346.

EDWARDS, R. W. 1958. The effect of larvae of *Chironomus riparius* Meigen on the redox potentials of settled activated sludge. Ann. Appl. Biol. 46: 457–464.

KAUSHIK, N. K., AND J. B. ROBINSON. 1976. Preliminary observations on nitrogen transport during summer in a small spring-fed Ontario stream. Hydrobiologia 49: 59–63.

KAUSHIK, N. K., J. B. ROBINSON, P. SAIN, H. R. WHITELEY, AND W. N. STAMMERS. 1975. A quantitative study of nitrogen loss from water of a small, spring-fed stream. Proc. Can. Symp. Water Pollut. Res., Toronto 10: 110–117.

KEENEY, D. R. 1973. The nitrogen cycle in sediment-water systems. J. Environ. Qual. 2: 15–28.

KIKUCHI, E., AND Y. KURIHARA. 1977. In vitro studies on the effects of tubificids on the biological, chemical and physical characteristics of submerged ricefield soil and overlying water. Oikos 29: 348–356.

PAINTER, H. A. 1977. Microbial transformations of inorganic nitrogen. Prog. Water Tech. 8: 3–29.

PATRICK, W. H. JR., R. D. DELAUNE, R. M. ENGLER, AND S. GOTOH. 1976. Nitrate removal from water at the water-mud interface in wetlands. Tech. Rep. No. EPA-600/3-76-042, 79 p.

ROBINSON, J. B. 1977. Modelling nitrogen transport and cycling in a natural flow system. Rep. Environ. Can. Inland Waters Direct. Ottawa, July 1977, 267 p.

ROBINSON, J. B., H. R. WHITELEY, W. N. STAMMERS, N. K. KAUSHIK, AND P. SAIN. 1979. The fate of nitrate in small streams and its management implications. Proc. 10th Annu. Cornell Univ. Conf., Rochester, N.Y., 247–259.

SAIN, P., J. B. ROBINSON, W. M. STAMMERS, N. K. KAUSHIK, AND H. R. WHITELEY. 1977. A laboratory study on the role of stream sediment in nitrogen loss from water. J. Environ. Qual. 6: 274–278.

STEEL, R. G. D., AND J. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill, New York, N.Y. 481 p.

TOMS, I. P., M. J. MINDENHALL, AND M. M. I. HARMAN. 1975. Factors affecting the removal of nitrate by sediments from rivers, lagoons and lakes. Tech. Rep. 14, Water Res. Centre, Stevenage, Herts., England.

VAN KESSEL, J. F. 1977. Removal of nitrate from effluent following discharge on surface water. Water Res. 11: 533–537.

WEST, P. W., AND T. P. RAMACHANDRAN. 1966. Spectrophotometric determination of nitrate using chromotropic acid. Anal. Chim. Acta 35: 317–324.

Carbon Isotope Ratios of Particulate Organic Matter in the Gulf of St. Lawrence

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TAN, F. C., AND P. M. STRAIN. 1979. Carbon isotope ratios of particulate organic matter in the Gulf of St. Lawrence. J. Fish. Res. Board Can. 36: 678–682.

Sixteen offshore surface samples within the Gulf of St. Lawrence show low $\delta^{13}C$ values and are similar to eight offshore surface samples collected seaward of the Gulf of St. Lawrence. The $\delta^{13}C$ surface values are consistent with $\delta^{13}C$ values in plankton produced at the temperature found in the euphotic zone in the study area. Higher values are observed in four surface samples from the mouth of the St. Lawrence Estuary and probably result from high carbon demand during periods of high biological productivity. Lower values found in seven deep POC samples indicate changes in the nature of the POC caused by biological degradation of the organic matter. Significant differences (2–6‰) between the uniformly high $\delta^{13}C$ values of the organic carbon in surface sediments and the low values of near-bottom water POC have been observed. The similarity between the $\delta^{13}C$ values of surface water POC and the surface sediments suggest that surface water POC is an important source of organic carbon in surface sediments. Several observations of large vertical $\delta^{13}C$ gradients in deep water POC suggest the presence of resuspended sediments 30–60 m above the sediment–water interface.

Key words: particulate organic matter, carbon isotope ratios, isotope fractionation, sediment resuspension, sediment sources, Gulf of St. Lawrence

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Seize échantillons d'eau superficielle du large prélevés à l'intérieur du golfe Saint-Laurent montrent de faibles valeurs de $\delta^{13}C$ et sont identiques à huit échantillons de surface recueillis en haute mer à l'extérieur du Golfe. Les valeurs de $\delta^{13}C$ de surface correspondent à celles de $\delta^{13}C$ dans le plancton produit à la température observée dans la zone euphotique de la région d'étude. Quatre échantillons prélevés à l'embouchure de l'estuaire du Saint-Laurent ont des valeurs plus élevées, résultat probable d'une forte demande en carbone durant des périodes de forte productivité biologique. Les valeurs inférieures trouvées dans sept échantillons de matière organique particulaire (POC) en profondeur révèlent des changements dans la nature de la POC causés par dégradation biologique de la matière organique. On observe des différences appréciables (2 à 6‰) entre les valeurs uniformément hautes de $\delta^{13}C$ du carbone organique dans les sédiments superficiels et les faibles valeurs de POC dans l'eau prélevée à proximité du fond. La similitude entre le $\delta^{13}C$ de la POC de l'eau superficielle et celui des sédiments super-

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ficiels donne à croire que la POC de l'eau de surface est une source importante de carbone organique dans les sédiments superficiels. Des gradients verticaux prononcés de $\delta^{13}C$ dans la POC d'eau profonde, observés à plusieurs reprises, suggèrent la présence de sédiments resuspendus à 30 à 60 m au-dessus de l'interface sédiment–eau.

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THIS note describes the distribution of $^{13}C:^{12}C$ ratios of particulate organic carbon (POC) in the Gulf of St. Lawrence and comments on the nature, stability, and degree of biodegradation of POC in various sectors of the coastal environment studied.

The distribution of total particulate matter in the Gulf of St. Lawrence has been reported by Sundby (1974), who also gives a brief outline of the oceanography of the study area. Previous studies on $^{13}C:^{12}C$ ratios in particulate organic matter in coastal environments include those of Calder (1969), Letolle and Martin (1970), Eadie and Jeffrey (1973), Haines (1976), and Schultz (1974).

Sampling and analytical methods — Water samples for POC analysis were collected with 12-L Niskin bottles during Bedford Institute Cruise 74-028 in July–August 1974. The precombusted Selas Flotronics® 0.8-μm silver membranes, which were used to separate the suspended particulate matter, were frozen at −20°C until analysis. In preparation for oxidation, the filters were loaded into precombusted porcelain combustion boats, covered with a few millilitres of high purity 10% HCl to remove inorganic carbonates, and evaporated to dryness at 50°C.

The POC samples were converted to CO_2 gas in a combustion system similar to that described by Craig (1953). The CO_2 produced was purified in a separate vacuum system containing Cu and MnO_2 for the removal of sulfur and nitrogen oxides (Sackett and Thompson 1963). The CO_2 yields were measured with a calibrated mercury manometer and $^{13}C:^{12}C$ ratios measured with a 15-cm, 60° sector ratio mass spectrometer (Nuclide Corporation). All the usual instrumental corrections were applied to the results (Deines 1970). The $^{13}C:^{12}C$ ratios are reported in the usual δ notation with respect to Pee Dee belemnite (PDB) standard. The precision (standard deviation of a single sample) of the analysis is estimated to be $\pm 0.6‰$ for the carbon isotope ratio and $\pm 7\ \mu g\ C\ L^{-1}$ for the concentration of organic carbon.

Results and discussion — Near surface and intermediate depths — Both the isotope ratio and the concentration of the POC exhibit a wide range within the study area indicated in Fig. 1. $\delta^{13}C$ ranges from −28.5‰ in the intermediate water in the central Gulf to −21.8‰ in surface water at the mouth of the St. Lawrence Estuary. Concentrations range from 14 $\mu g\ L^{-1}$ in intermediate water in Cabot Strait to 770 $\mu g\ L^{-1}$ in surface water in Hermitage Channel on the south coast of Newfoundland.

Tables 1 and 2 summarize the POC isotope ratios and concentrations, respectively, for five different types of sampling environments in the study area. Since little is known about the nature of the POC–$\delta^{13}C$ distribution, arithmetic means (i.e. normal distributions) are used in Table 1. However, POC concentrations are known to have a log-normal distribution (e.g. Wangersky 1976); therefore, geometric means are used in Table 2. Statistics for surface samples are reported separately for four different areas: (a) the regions of the Gulf of St. Lawrence removed from immediate land influence, (b) two coastal inlets — namely Hermitage Channel and Bonne Bay, (c) areas seaward of the Gulf of St. Lawrence, and (d) the mouth of the St. Lawrence Estuary. The fifth set of data is for deep-water samples from the Laurentian Channel and its branches. Included in this group are samples from water whose temp ≥ 3.8°C and $S \simeq 34.5‰$. Excluded are samples from nearshore stations and samples that are believed to have been influenced by the resuspension of surface sediments.

The isotope ratios of surface water POC, with the exception of the mouth of the St. Lawrence Estuary, are consistently close to −25.0‰. This value, taken in context with the related isotopic and oceanographic information, can be used to comment on the nature of the POC in the following way.

Sackett et al. (1965) and Deuser et al. (1968) have shown that plankton sampled at the observed surface

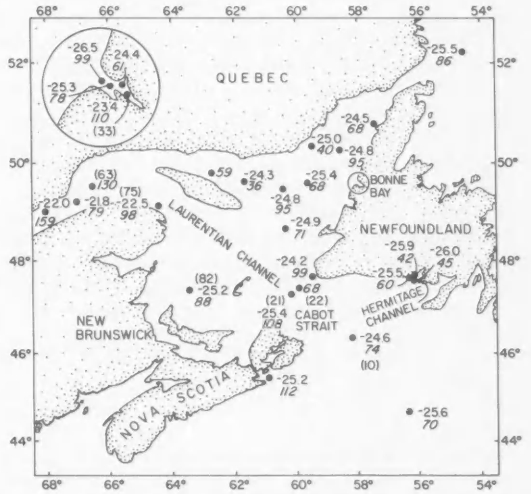

FIG. 1. Gulf of St. Lawrence — study area showing sampling locations. Normal and italicized numbers are the mean $\delta^{13}C_{PDB}$ values (‰) and concentrations ($\mu g\ L^{-1}$), respectively, of surface layer (upper 50 m) POC. Numbers in parentheses are station numbers.

TABLE 1. Arithmetic mean, standard deviation, range, and number of POC isotope ratios for five different sampling environments in the study area.

	Mean (‰)	σ (‰)	Range (‰)	n
Gulf of St. Lawrence (upper 50 m)	−24.9	0.5	1.8	16
Coastal inlets (upper 50 m)	−25.1	1.4	4.9	9
Outside Gulf (upper 50 m)	−25.2	0.9	2.4	8
Mouth of St. Lawrence Estuary (upper 50 m)	−22.4	0.7	1.6	4
Laurentian Channel water (S ≃ 34.5‰, temp ≥ 3.8°C)	−26.2	1.0	2.9	7

temperatures (mean temperature = 11.4°C) from water with a $\delta^{13}C$ of 2.1‰ for dissolved inorganic carbon (Tan and Walton 1978) have a $\delta^{13}C$ organic carbon value close to −25.0‰. This isotope ratio also falls between the $\delta^{13}C$ plankton range (−29.2 to −25.6‰) reported for Antarctic surface waters with temperatures of −1.4 to −0.4°C (Eadie and Sackett 1971) and the range (−20.5 to −17.8‰) observed for plankton from warm Gulf of Mexico surface waters (Eadie 1972). Since the Gulf of St. Lawrence samples were collected in July and August shortly after the spring bloom (Steven 1974), it is reasonable to assume that the POC consists predominantly of living material or recently dead organisms. It is conceivable that the complex estuarine circulation of the study area could produce POC residence times long enough to allow significant biological degradation of organic detritus. However, such degraded material should have an isotope ratio different from the expected plankton isotope ratio due to the selective removal of isotopically heavier biochemicals such as amino acids and sugars (Eadie and Jeffrey 1973).

The surface POC isotope ratio is also similar to the isotope ratio found for organic carbon of terrestrial origin in the surface sediments of the St. Lawrence River (−25.4‰) and the Saguenay Fjord (−25.9‰) (Tan and Strain 1979). However, since virtually all terrestrial organic carbon is shown to be deposited within the St. Lawrence Estuary and other nearshore areas (Tan and Strain 1979; Pocklington 1976), the contribution of terrestrial organic matter to open Gulf surface water POC is probably negligible.

We conclude, therefore, that the surface POC found in our study consists mostly of recent in situ-produced organic material.

The four surface POC samples collected from the mouth of the St. Lawrence Estuary have the highest $\delta^{13}C$ values (−22.4‰) observed in the entire study area. They are significantly heavier ($P < 0.01$ in the least significant case) than the other groups of surface samples. If the organic–inorganic isotope fractionation of carbon is considered, the difference between the behavior in the Estuary and in the other parts of the study area is even greater. The $\delta^{13}C$ of the total dissolved inorganic carbonate in the surface water of the Gulf of St. Lawrence is +2.1‰ (Tan and Walton 1978). In the mouth of the St. Lawrence Estuary, this value varies seasonally but is generally about 2‰ lower than in the Gulf (Tan and Strain unpublished data). The $\delta^{13}C$ differences between the POC and the total dissolved CO_2 are therefore 22‰ for the St. Lawrence Estuary and 27‰ for the remainder of the study area.

It is conceivable that this difference is a result of a nonuniform distribution of planktonic species in the study area. However, Steven (1974) did not note any significant differences between the plankton from the two areas. The fact that Steven did note that productivity is significantly higher at the mouth of the Estuary than in the open Gulf suggests an alternate explanation — namely that high carbon demand is responsible for the high carbon isotope ratio.

Deuser et al. (1968) used laboratory culture experiments to show that plankton, which use dissolved CO_2 rather than HCO_3^- in photosynthesis, can use the available CO_2 quickly enough at times of high productivity to cause an isotopic (but not chemical) disequilibrium between the dissolved CO_2 and the HCO_3^-. Since HCO^{3-} carbon has ~7–9‰ more ^{13}C than dissolved CO_2 carbon, the organic carbon produced in nonequilibrium conditions will be unusually heavy.

Deuser (1970) has observed such behavior in the Black Sea. Since we believe the surface water POC consisted mostly of isotopically unaltered living material at the time of sampling, we believe that high

TABLE 2. Geometric mean, standard deviation, range, and number of POC concentrations for five different sampling environments in the study area.

	$\bar{x}$ (μg/L)	$\bar{x} + \sigma$ (μg/L)	$\bar{x} - \sigma$ (μg/L)	Range (μg/L)	n
Gulf of St. Lawrence (upper 50 m)	65	115	37	167	19
Coastal inlets (upper 50 m)	93	215	40	729	11
Outside Gulf (upper 50 m)	82	110	61	87	8
Mouth of St. Lawrence Estuary (upper 50 m)	96	150	62	108	5
Laurentian Channel water (S ≃ 34.5‰, temp ≥ 3.8°C)	27	35	21	24	8

TABLE 3. POC trends near the sediment–water interface. $\delta^{13}C_1$, $\delta^{13}C_2$:POC isotope ratios in bottom water—subscript 2 indicates the sample closer to the sediment. $[POC]_1$, $[POC]_2$ indicate POC concentrations at the depths corresponding to $\delta^{13}C_1$ and $\delta^{13}C_2$. $\delta^{13}C_3$ is the organic carbon isotope ratio in the surface sediment (Tan and Strain 1979).

Station	$\delta^{13}C_1-\delta^{13}C_2$ (‰)	$[POC]_1-[POC]_2$ (μg/L)	$\delta^{13}C_2-\delta^{13}C_3$ (‰)
10	−1.0	−24	−4.4
22	−2.1	−3	−2.2
33	−1.6	+47	−2.9
63	−1.0	−14	−2.8
75	+1.3	−20	−3.8
82	−0.2	−92	−2.5

carbon demand is responsible for the heavy POC observed in the mouth of the St. Lawrence Estuary.

Deep water — Deep Laurentian Channel POC is significantly different in isotope ratio from POC in the upper 50 m of the open Gulf ($P < 0.001$), and from POC in the upper 50 m of areas outside the Gulf ($P < 0.05$). In addition, the POC concentrations in the deep Laurentian Channel water are lower than those found in the four groups of surface samples ($P < 0.005$ in the least significant case). The absence of large living particles in the deep water leads to these low POC concentrations. The isotope ratios of the near-bottom water POC are also different from those of the organic carbon in the surface sediments (see Table 3 and Fig. 2, 3). The $\delta^{13}C$ values of organic carbon in surface sediments underlying the central Gulf fall in a narrow range of $-22.4 \pm 0.2(1\sigma)$‰ (Tan and Strain 1979) compared to -24.9 ± 0.5 for surface water POC and -26.2 ± 1.0 for deep-water POC. The difference between surface and near-bottom water POC, which is readily seen in the profiles shown in Fig. 2–3, is consistent with the possibility that the latter is mostly composed of small particles with long residence times. The organic carbon in such particles would be significantly altered, leaving only the refractory biochemical fractions (Eadie and Jeffrey 1973).

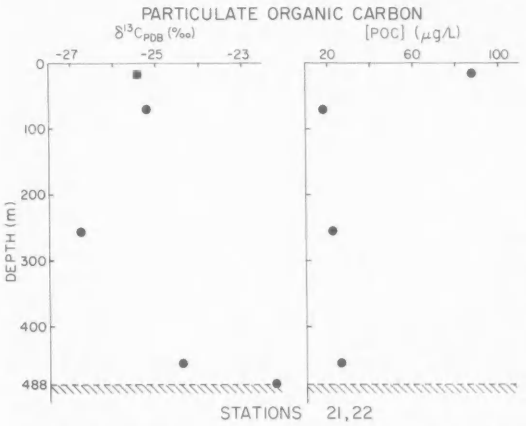

FIG. 2. Composite vertical profiles of particulate organic carbon isotope ratio and concentration for stations 21 and 22.

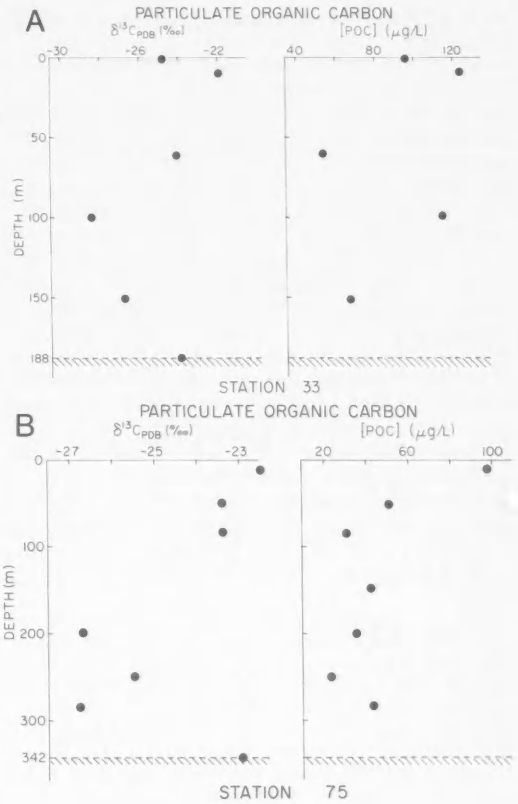

FIG. 3. Vertical profiles of particulate organic carbon isotope ratio and concentration for A) station 33 and B) 75.

The difference between the $\delta^{13}C$ values of the near-bottom water POC and the organic carbon in the surface sediments has two possible explanations. Either this POC undergoes rapid diagenesis following deposition or it is only a minor contributor of organic carbon to the surface sediments. The first suggestion is considered unlikely in view of the expected refractory nature of the near-bottom water POC. The second explanation is more consistent with the idea that large particles such as fecal pellets which originate in the surface layer and fall rapidly through the water column are the dominant source of sedimentary material (McCave 1975). The concentration of these large particles at depth is so low that the probability of their occur-

rence in small volume samples is low. The $\delta^{13}C$ profiles for stations 33 and 75 (Fig. 3) show that the $\delta^{13}C$ values of the surface sediments are sometimes closer to that of surface water POC than to that of near-bottom water POC. Thus we feel that only a small portion of the organic carbon in these surface sediments originates from the "standing crop" of near-bottom water POC.

The isotopic difference between the deep-water POC and the sedimentary organic carbon gives rise to gradients in POC $\delta^{13}C$ values near the sediment–water interface. Table 3 gives the changes in $\delta^{13}C$ values and concentrations in deep-water POC near the sediment for six stations; trends are shown in Fig. 2 and 3 for three of these stations. At five of the six stations, the POC $\delta^{13}C$ values become more positive and the concentrations higher in the water adjacent to the sediment. Both these observations strongly suggest that the standing crop of deep-water POC is being mixed with organic carbon resuspended from surface sediments. It is possible that more detailed POC $\delta^{13}C$ vertical profiles could be used to measure the extent of resuspension in situations in which the isotope ratio of the standing crop of deep-water POC can be measured.

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CALDER, J. 1969. Carbon isotope effects in biochemical and geochemical systems. Ph.D. thesis, Univ. Texas, Austin, Tex. 129 p.

CRAIG, H. 1953. The geochemistry of the stable carbon isotopes. Geochim. Cosmochim. Acta 3: 53–92.

DEINES, P. 1970. Mass spectrometer correction factors for the determination of small isotopic composition variations of carbon and oxygen. Int. J. Mass Spectrom. Ion Phys. 4: 283–295.

DEUSER, W. G. 1970. Isotopic evidence for diminishing supply of available carbon during diatom bloom in the Black Sea. Nature 225: 1069–1070.

DEUSER, W. G., E. T. DEGENS, AND R. R. L. GUILLARD. 1968. Carbon isotope relationships between plankton and sea water. Geochim. Cosmochim. Acta 32: 657–660.

EADIE, B. J. 1972. Distribution and fractionation of stable carbon isotopes in the Antarctic Ecosystem. Ph.D. thesis, Texas A and M Univ., College Station, Tex. 110 p.

EADIE, B. J., AND L. M. JEFFREY. 1973. $\delta^{13}C$ analyses of oceanic particulate organic matter. Mar. Chem. 1: 199–209.

EADIE, B. J., AND W. M. SACKETT. 1971. Stable carbon isotope variations in the Antarctic marine ecosystem. Antarct. J. U.S. 6: 154.

HAINES, E. B. 1976. Stable carbon isotope ratios in the biota, soils and tidal water of a Georgia salt marsh. Estuarine Coastal Mar. Sci. 4: 609–616.

LETOLLE, R., AND T. M. MARTIN. 1970. Carbon isotope composition of suspended organic matter in two European estuaries. Mod. Geol. 1: 275–278.

MCCAVE, I. N. 1975. Vertical flux of particles in the ocean. Deep-Sea Res. 22: 491–502.

POCKLINGTON, R. 1976. Terrigeneous organic matter in surface sediments from the Gulf of St. Lawrence. J. Fish. Res. Board Can. 33: 93–97.

SACKETT, W. M., W. R. ECKELMANN, M. L. BENDER, AND A. W. H. BE. 1965. Temperature dependance of carbon isotope composition in marine plankton and sediments. Science 148: 235–237.

SACKETT, W. M., AND R. R. THOMPSON. 1963. Isotopic organic carbon composition of Recent continental derived clastic sediments of east Gulf of Mexico. Bull. Am. Assoc. Pet. Geol. 47: 525.

SCHULTZ, D. J. 1974. Stable carbon isotope variations in organic and inorganic carbon reservoirs in the Fenholloway River Estuary and the Mississippi River Estuary. Ph.D. thesis, Florida State Univ., Tallahassee, Fla. 162 p

STEVEN, D. M. 1974. Primary and secondary production in the Gulf of St. Lawrence. McGill Univ. Mar. Sci. Center MS Rep. 26: 116 p.

SUNDBY, B. 1974. Distribution and transport of suspended particulate matter in the Gulf of St. Lawrence. Can. J. Earth Sci. 11: 1517–1533.

TAN, F. C., AND P. M. STRAIN. 1979. Organic carbon isotope ratios in recent sediments in the St. Lawrence Estuary and the Gulf of St. Lawrence. Estuarine Coastal Mar. Sci. (In press)

TAN, F. C., AND A. WALTON. 1978. Stable isotope studies in the Gulf of St. Lawrence, Canada. *In* Stable isotopes in the earth sciences, DSIR Bulletin 220, New Zealand, 229 p.

WANGERSKY, P. J. 1976. Particulate organic carbon in the Atlantic and Pacific oceans. Deep-Sea Res. 23: 457–465.

Composition of Granules from Kidneys of Rainbow Trout (*Salmo gairdneri*) with Nephrocalcinosis

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GILLESPIE, D. C., AND R. E. EVANS. 1979. Composition of granules from kidneys of rainbow trout (*Salmo gairdneri*) with nephrocalcinosis. J. Fish. Res. Board Can. 36: 683–685.

Histological sections and isolated granules from kidney tubules of rainbow trout (*Salmo gairdneri*) with nephrocalcinosis show that the mineral deposits form a network imbedded in a ceroid matrix. The mineral portion, about 80% by weight of the granules, contains 30% Ca, 2.5% Mg, 15% P, and 5% CO_3. The matrix is basophilic, sudanophilic and acid fast, is insoluble in lipid solvents but will dissolve in hot 10% NaOH.

Key words: nephrocalcinosis, calcification, ceroid, rainbow trout

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Des préparations histologiques et des granules isolées des tubules rénaux de truites arc-en-ciel affectées de néphrocalcinose indiquent que les dépôts minéraux forment un réseau enrobé dans une matrice céroïde. La portion minérale, qui est d'environ 80% en poids des granules, contient 30% de Ca, 2,5% de Mg, 15% de P et 5% de CO_3. La matrice est basophile, sudanophile et acido-résiatante, est insoluble dans les solvents de lipides, mais se dissout dans le NaOH à 10% chaud.

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WE found lesions in the kidneys of rainbow trout (*Salmo gairdneri*) from the Rockwood Hatchery of the Freshwater Institute with a gross appearance similar to those reported by Wunder (1967) in fish that had "nephrocalcinosis." The disease is characterized by renal deposits high in calcium and is of noninfectious but unknown etiology (Landolt 1975). Herman (1971) describes the disease as a chronic, inflammatory disease that generally does not result in high mortality. The renal deposits are accompanied by tubular dilation and degeneration, the formation of tubular casts, and in extreme cases, the destruction of most of the functional kidney tissue (Landolt 1975). We felt that an analysis of the granules' chemical composition would supply clues as to how the renal deposits were formed. The present report describes the composition and structure of granular deposits associated with nephrocalcinosis of rainbow trout.

Methods — Pooled kidney lesions were shredded with forceps and suspended in distilled water. The granules were separated from kidney tissue by vigorous stirring and decanting the supernatant fluid. The process was repeated until the supernatant fluid was clear and no tissue could be seen microscopically. The granules were washed with 95% ethanol, subsequently with acetone and air-dried.

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Granule samples were exposed to 0.1 N HCl, 1 N HCl, 1 N H_2SO_4, 1 N NaOH, and several lipid solvents (ethanol, acetone, ether). Some kidney granules were extracted with boiling 10% NaOH (Endicott and Lillie 1944).

Chemical analysis for Mg and Ca was carried out by Flame Atomic Absorption Spectrophotometry. A sample consisted of 50 mg of dry ash dissolved in nitric acid to a volume of 25 mL. The calcium sample included 1.25 mL of $LaCl_2$ to prevent interference due to phosphorus. Analysis for P was done by the Molybdenum Blue Method of Murphy and Riley (1962). Carbonates were determined in granule samples by the method of Stainton (1973).

Whole kidneys were fixed in Bouin's fluid, sectioned, and stained with 0.5% aqueous toluidine blue, sudan black B in 70% ethanol (Thompson 1966), and the Ziehl–Neelson carbol fuchsin stain (Pearse 1972). Kidneys were also fixed in 10% neutral formalin and stained for calcium with Alizarin Red S (Thompson 1966).

Results — Air-dried granules were buffy-yellow and varied in size from ~0.1 to 2.0 mm. The granules contained 19.3% Ca, 2.3% Mg, 13.1% P, and 4.9% carbonates. The yellow residue after treatment with 0.1 N HCl or the yellow portion of untreated granules was insoluble in 1 N HCl, 1 N H_2SO_4, and 1 N NaOH and had a waxy consistency. The substance was organic since it charred when heated; its insolubility in a large number of lipid solvents indicated that it was probably ceroid (Wood and Yasutake 1956).

Extraction of kidney granules with boiling 10% NaOH resulted in no obvious morphological change in the granules (Fig. 1) but following this treatment they

were pure white in color and dissolved completely with effervescence in dilute HCl. Chemical analysis of the NaOH extracted granules gave a composition of 30.3% Ca, 2.5% Mg, 15.3% P, and 2.5% CO_3.

Neutralization of the NaOH extract produced a flocculent precipitate that, under the microscope, resolved into small yellow–brown grobules in clusters intermingled with a few yellow–brown needles. This material was not acid fast and represented degraded ceroid.

Bouin-fixed histological kidney sections showed granules in renal tubules that were morphologically the same as isolated ones (Fig. 2). These granules were basophilic, sudanophilic, and acid fast. Calcium within the granules stained with Alizarin Red S in kidney tissue fixed with 10% neutral formalin.

Isolated granules were positive for Ca by Alizarin Red staining before but not after treatment with dilute HCl. They were sudanophilic and acid fast before hot NaOH extraction, but not after, and retained positive calcium staining. The dilute HCl residue was sudanophilic and acid fast (Fig. 3) and comprised a network of ceroid material with empty spaces where the Ca–Mg salts had been dissolved away.

Discussion — The Ca:P ratio of 2:1 in both extracted and unextracted granules was consistent with tricalcium phosphate being the major mineral component. Only 80% of the weight of the NaOH-extracted granules was accounted for. The remainder could be other metals since there was an excess of CO_3 over Mg, or it could be water of dehydration. Robbins (1974) describes kidney "stone" crystalloids with the following frequency: 90% of stones contain calcium, 70% contain oxalate, 5–10% contain urates, and 2–3% contain cystine. The majority of concentrically arranged stones contain mixtures of these crystalloids with small amounts of magnesium and sodium. Calcium oxalate is one of the most common compounds in human urinary calculi. About half of all stones contain phosphate. Five different phosphates occur in renal calculi. Two of these, apatite (complex calcium phosphates) and magnesium ammonium phosphate, form the great bulk of the material. Chemical analysis of calculi containing apatites is generally reported as showing "calcium phosphate" and calcium carbonate assuming that the former is tricalcium phosphate (Prien and Prien 1968). Trout nephrocalcinosis granules and some human urinary stones have chemical constituents similar to those of bone.

The staining properties of the renal granule matrix and its insolubility in various lipid solvents suggests that it is ceroid. The difference of about 15% in recovery between NaOH-extracted and untreated granules could represent ceroids; this calculation is supported by the fact that 13% of the granules was insoluble in 1 N HCl. The mineral/ceroid ratio probably varies from

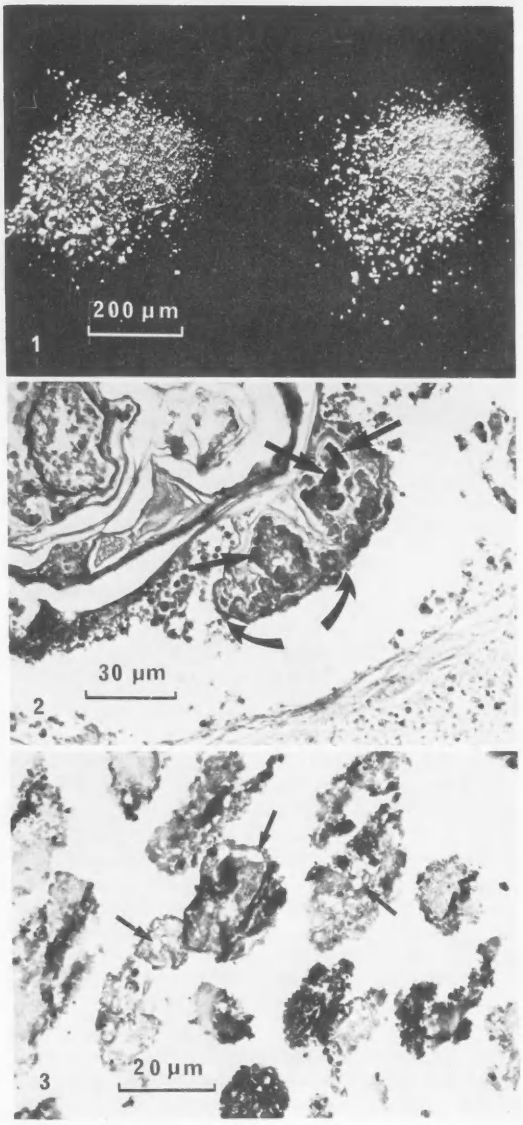

FIG. 1. *Left*, unextracted granules removed from trout with nephrocalcinosis. *Right*, kidney granules extracted with 10% NaOH; FIG. 2. Nephrocalcinosis granules within a degenerated renal tubule, fixed in Bouins fluid, secioned at 6 µm. Stain: Ziehl–Neelson (acid fast). Straight arrows indicate the calcified mineral deposits within the granules. Curved arrows indicate the extremities of one granule. FIG. 3. Acid-extracted, paraffin-embedded nephrocalcinosis granules sectioned at 6 µm. Stain: Sudan Black B in 70% ethanol. Arrows indicate spaces occupied by mineral deposits before acid extraction.

fish to fish since microscopic examination showed a wide variation in particle size. In some cases numerous particles without ceroid coating were also observed.

Ceroids are pigments probably formed by the auto-oxidation of products of unsaturated fats, fatty acids, or their esters. Since fish have a naturally high content of unsaturated fatty acids, they are particularly susceptible to ceroid formation. Ceroid is not normally present in fish; however, nutritional deficiencies, bacterial and virus diseases, toxic products, and other physiologic disturbances readily result in the formation of the pigments (Wood and Yasutake 1956). Cellular necrosis is an important etiological agent in ceroid production (Wood and Yasutake 1956). Necrosis and ceroid were observed in trout nephrocalcinosis. Perhaps the breakdown of cells destroys a biochemical mechanism that prevents auto-oxidation of the unsaturated fatty acids, allowing the formation of ceroid. Dead or degenerating tissues often become impregnated with deposits of calcium salts without an abnormal serum calcium level. Therefore necrotic tissues in nephrocalcinosis are probably responsible for the calcified deposits in the ceroid matrix. What causes the initial kidney inflammation and necrosis remains unknown.

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ENDICOTT, K. M., AND R. D. LILLIE. 1944. Ceroid, the pigment of dietary cirrhosis of rats. Its characteristics and its differentiation from hemofuscin. Am. J. Pathol. 20: 1119–1153.

HERMAN, R. L. 1971. Visceral granuloma and nephrocalcinosis. U.S. Bur. Sport Fish. Wildl. Fish. Dis. Leafl. 32.

LANDOLT, M. L. 1975. Visceral ganuloma and nephrocalcinosis of trout, p. 793–800. *In* W. A. Ribelin and G. Migaki [ed.] The pathology of fishes. University of Wisconsin Press, Madison, Wisc.

MURPHY, J., AND J. RILEY. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27: 31–36.

PEARSE, A. G. E. 1972. Histochemistry: theoretical and applied. 3rd ed. Vol. 2. Churchill Livingstone, London.

PRIEN, E. L., AND E. L. PRIEN JR. 1968. Composition and structure of urinary stone. Am. J. Med. 45: 654–672.

ROBBINS, A. L. 1974. Pathologic basis of disease. W. B. Saunders Company, Philadelphia, London, Toronto.

STAINTON, M. P. 1973. Syringe gas-stripping procedure for gas-chromatographic determination of dissolved inorganic and organic carbon in freshwater and carbonates in sediments. J. Fish. Res. Board Can. 30: 1441–1445.

THOMPSON, S. W. 1966. Selected histochemical and histopathological methods. C. C. Thomas, Springfield, Ill.

WOOD, E. M., AND W. T. YASUTAKE. 1956. Ceroid in fish. Am. J. Pathol. 32: 591–603.

WUNDER, W. 1967. Eigenartige Veranderungen an der Niere und die Ausbildung einer Nierenzyste bei der Regenbogenforelle. Allg. Fisch-Ztg. 95: 470–471. (Fish. Mar. Serv. Transl. Ser. No. 3196)

Rate of Loss of Ammonia from Water to the Atmosphere

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WEILER, R. R. 1979. Rate of loss of ammonia from water to the atmosphere. J. Fish. Res. Board Can. 36: 685–689.

The rate of loss of ammonia from water to the atmosphere has been measured both in the field and in the laboratory as a function of wind speed, temperature, and pH. The exchange coefficient, K_e, was found to be a linear function of wind speed and temperature. Although the loss rates (1–10 $kg \cdot ha^{-1} \cdot d^{-1}$) are quite high at pH 9 and at ammonia concentrations >1 mg $N \cdot dm^{-3}$, the rates are much smaller under the conditions found in most natural water bodies.

Key words: ammonia, exchange coefficient, rate of loss from water

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Nous avons mesuré la vitesse de perte d'ammoniac de l'eau vers l'atmosphère, sur le terrain aussi bien qu'en laboratoire, en fonction de la vitesse du vent, de la température et du pH. Nous constatons que le coefficient d'échange, K_e, est une fonction linéaire de la vitesse du vent et de la température. Bien que les taux de perte (1 à 10 $kg \cdot ha^{-1} \cdot d^{-1}$) soient plutôt élevés à un pH de 9 et à des concentrations d'ammoniac supérieures à 1 mg $N \cdot dm^{-3}$, les taux sont beaucoup moindres dans les conditions rencontrées dans la plupart des masses d'eau.

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RASMUSSEN et al. (1975) have discussed the natural and man-made sources and sinks for ammonia. Bacterial decomposition of organic material on the earth's surface appears to be the primary source of atmospheric NH_3. Probably the most important removal mechanism is its solution in rainwater together with sulphur dioxide or other gases to form aerosols, with up to 75% removed by rainout and washout. It is not clear whether the ocean acts as a source or sink for ammonia because the available data can be interpreted both ways (Junge 1963).

Hutchinson and Viets (1969) measured the uptake of ammonia in dilute acid and water traps. Calder (1972) developed a simple mathematical model to estimate the fraction of ammonia lost from a cloud travelling over an absorbing water face. Stratton (1968, 1969) studied the loss of ammonia from artificial streams (flumes) and from small floating enclosures in alkaline lakes.

The impetus for undertaking this investigation came from trying to establish the magnitude of air–water exchange of ammonia as a term in the nitrogen budget of a small eutrophic lake. The rate of loss of ammonia from water was measured as a function of temperature, wind speed, and pH both in the field and in the laboratory. From the rate, the exchange coefficient was calculated.

Methods — The ammonia concentration in the water was measured by the alkaline phenol method with a Technicon Auto Analyzer (Philbert and Traversy 1973). The detection limit is 1 mg $N \cdot m^{-3}$. The standards for lake water samples were made up in deionized water. When borax buffer (0.05 $mol \cdot dm^{-3}$ $Na_2B_4O_7 \cdot 10H_2O$; pH 9.2) was used as the experimental fluid, the standards were also made up in the buffer. For the carbonate–biocarbonate buffer (0.025 $mol \cdot dm^{-3}$ $NaHCO_3$ + 0.025 $mol \cdot dm^{-3}$ Na_2CO_3; pH 10), the samples and standards were diluted 5:1 with sufficient 1.3% HCl to bring the pH down to 6.5 before analysis.

The field experiments were done in Lake St. George (43°57′1″N; 79°25′18″W), a small lake near Maple, north of Toronto. The lake is made up of two basins, each ~200–250 m diam and surrounded by trees. The water is fairly hard, with a total inorganic carbon content of ~2 $mol \cdot m^{-3}$.

A raft for equipment was moored near the middle of the larger basin. Some of the experiments were done in a minicorral ~81 cm diam, made of polyethylene and supported by floats beside the raft. It contained ~600 dm^3 of tap water poisoned with $HgCl_2$ to reduce biological activity. Its top could be adjusted to float <5 cm above the lake surface. Circulation in the corral was maintained by a peristaltic pump taking in water at the surface and discharging it at the bottom.

The other experimental enclosure, a rectangular plastic container 54 × 35 × 25 cm deep, sat on the raft. It was filled to within 2 cm of the top with either borate or bicarbonate buffer. Sufficient NH_4Cl was added to both vessels at the start of the experiment to bring the concentration in the range of 800–1000 mg $N \cdot m^{-3}$.

Samples for analysis were collected at the surface in small polyethylene bottles and analyzed within 15–30 min on the Auto Analyzer system located in a trailer on the site. At the same time, the temperature was measured with a thermometer and the pH with a combination electrode. The wind was measured with an anemometer located 4 m above the lake surface.

The floor of a small plexiglass wind tunnel (Lau, CCIW, 1976, unpublished data), 125 cm long with a cross section of 15 × 30.5 cm was cut away at the test section so that a basin 27.5 × 27.5 × 15.0 cm deep could be fitted directly underneath. The water level in the basin was ~2.5 cm below the floor.

Air was drawn in by a fan through a bell-shaped entrance and a bank of flow straighteners and exhausted to the room (Fig. 1). The air velocity, which can be varied between 1 and 7 $m \cdot s^{-1}$, was measured by a pilot tube located 6 cm above the floor of the tunnel in a region of reasonably uniform velocity. A stirrer turning at ~25 rpm was sufficient to induce a clockwise circulation (viewed from top) in the tank, but not enough to create a vortex on the surface. A metal coil connected to a small constant temperature, circulating bath maintained the water temperature to within ±0.1 deg C for runs at or close to 20°C. Runs at ~10°C showed a slow upward drift of ~0.2 $deg \cdot h^{-1}$. About 10 min before a sample was taken, the water level was adjusted to the starting level by adding deionized water through a narrow plastic tube taped to the thermometer.

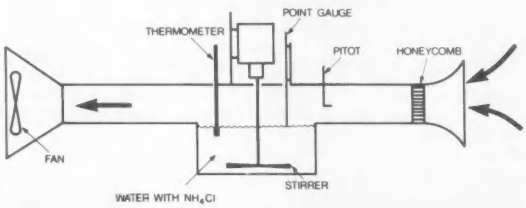

FIG. 1. Schematic sketch of wind tunnel (not to scale).

Therefore, no corrections had to be made for concentration changes caused by evaporation, but allowance had to be made for the water withdrawn for sampling. Samples were removed with a syringe through the same tube and analyzed immediately.

At the start of a run, the buffer solutions (borate or bicarbonate) were spiked with enough NH_4Cl to bring the starting concentration to 800–1000 mg $N \cdot m^{-3}$.

A run lasted from 6 to 10 h and samples were taken at ~1-h intervals. The air temperature in the room varied between 21 and 24°C.

In calculating the exchange rate coefficient, it is assumed that the loss of nitrogen from a well-stirred vessel is entirely a function of the un-ionized ammonia concentration in bulk solution. The rate of loss of nitrogen (N) is then proportional to the NH_3 concentration (Blain 1969), that is

$$\frac{d[N]}{dt} = -K[NH_3] \tag{1}$$

where K is the overall loss coefficient. A more fundamental measure of the rate is the mass-transfer coefficient or exchange coefficient, which is defined by Holley (1977) as

$$K_e = \left(\frac{V}{A}\right)K \tag{2}$$

and has the units length/time. A is the area and V the volume of the vessel. Because

$$[N] = [NH_3] + [NH_4^+] \tag{3}$$

and

$$K_a = \frac{[H^+][NH_3]}{[NH_4^+]}, \tag{4}$$

Equation (1) can be written in terms of N as follows:

$$\frac{d[N]}{dt} = -K_e\left(\frac{A}{V}\right)\frac{[N]}{(1+[H^+]/K_a)}, \tag{5}$$

integrating

$$[N] = [N_o]\exp -\left\{\frac{K_e A t}{V(1+[H^+]/K_a)}\right\}. \tag{6}$$

In this formulation, K_e is independent of the pH, but $d[N]/dt$ is not.

In practice, ln N was plotted against t, a least mean square line was fitted, and, from the slope, K_e was calculated. The correlation coefficient was >0.97 for the laboratory runs and >0.9 for the runs done in the field.

The value for K_a was taken from Emerson et al. (1975). $[H^+]$ and K_a had constant values for the wind-tunnel experiments. For the field experiments, the measured pH and temperature values were used. Hence, K_a varied during the run.

The K_e values can be converted to K values by multiplying with the factor A/V which has the following values: minicorral: 8.7×10^{-3}; container on raft: 0.046; wind tunnel: 0.069. The value of K_e or K at a particular pH and temperature can be found by dividing by $(1 + [H^+]/K_a)$.

Results — The results for variation of K_e with wind speed are shown in Fig. 2. The least means square line fitted to the data is

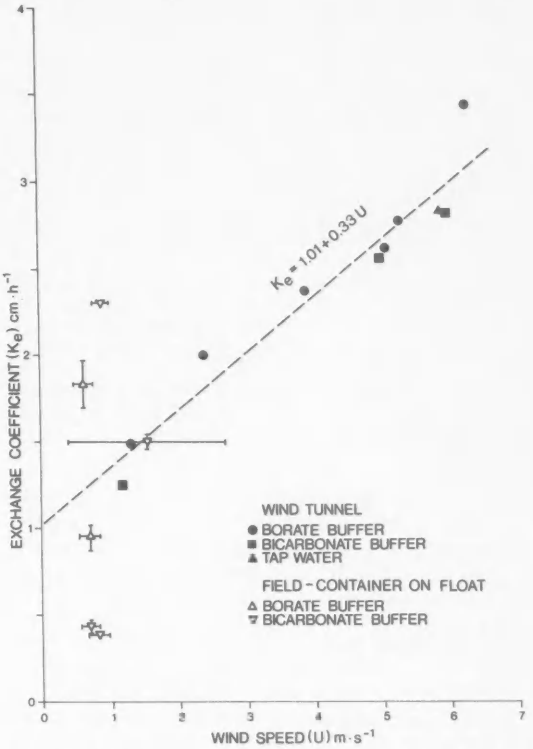

FIG. 2. Variation of exchange coefficient, K_e with wind speeds as measured in the wind tunnel and in the field. Error bars represent 1 SD.

$$K_e = 1.01 + 0.33\,U \tag{7}$$

where K_e is in $cm \cdot h^{-1}$ and U, the wind speed, in $m \cdot s^{-1}$.

The wind speed varied by ~1–2% during a run. The pH measurements varied by ~±0.02 units and the temperature by <±0.1 deg C. The term $(1 + [H^+]/K_a)$ in equation (5) is much more sensitive to changes in pH than to changes in temperature. At pH 9.2, a change from 20 to 21°C or 10 times the observed variation during a run changes pK_a from 9.40 to 9.37 and the term from 1.63 to 1.68 or ~3%. However, a change from pH 9.18 to pH 9.22 at 20°C, which is the observed uncertainty during a run, changes the term from 1.60 to 1.66 or ~3.6%.

The results for the variation of K_e with temperature are shown in Fig. 3. The runs were done in borax buffer at a wind speed of ~5.2 $m \cdot s^{-1}$.

To obtain an estimate of the error introduced by the slow temperature drift at the lower temperature, I calculated a K_e value from the linear ln [N] vs. t plot at each sampling time, using the K_a corresponding to the measured temperature and the theoretical pH of the

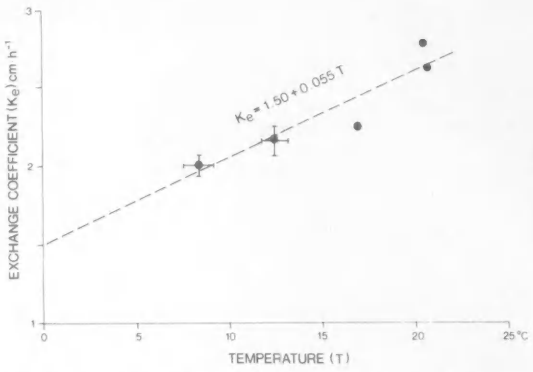

FIG. 3. Variation of exchange coefficient, K_e, with temperature as measured in the wind tunnel at a wind speed of 5.2 m·s^{-1}. Error bars represent 1 SD.

buffer at the same temperature. The measured K_e's at 8.3 and 12.4°C in Fig. 3 are therefore the averages of all of the individual K_e's for that run.

The K_e values for the field experiments using the minicorral are listed in Table 1. The results using the plastic container on the raft are shown in Fig. 2. The wind speeds are those measured at 4 m above the lake.

Discussion — The results obtained in the field using the container on the raft are within a factor of 2–3 of the wind-tunnel results whereas those obtained using the minicorral are about an order of magnitude higher. The field results are also much more variable than the wind tunnel results.

Some of this variability can be ascribed to the unavoidable variations in the field of temperature, pH, and wind speed. A small error is introduced by the fact that the wind was measured at 4 m in the field but at 6 cm in the laboratory. The results obtained by Roll (1965) show that at low speeds (<2 m·s^{-1}), the wind speed at 6 cm is ~20–30% lower than the speed at 4 m.

The reasons for the larger values obtained from the minicorral measurements (Table 1) are not known. Because the larger surface area of the corral, the wind-induced stirring is probably more efficient. Certainly larger ripples appeared on it than on the container on the raft. Although the water in the corral was circulated by means of a pump, it is possible that concentration gradients developed. In that case, perhaps only the top layer and not all of the volume should be considered. As is clear from equation (5), if V is made smaller, then K_e is also smaller.

It is also possible that other processes besides exchange were affecting the ammonia concentrations in the corral. Although the water was poisoned with $HgCl_2$, there is no guarantee that the water remained sterile. During the first part of the run (~8 h) the ammonia concentration decreased. However, the concentration the next morning had not changed from the evening before or had even increased, although this effect was also noticed on occasion in the container on the float.

TABLE 1. Variation of K_e with wind speed in minicorral.

Date 1976	Wind speed[a] (m·s^{-1})	Temp °C	pH	K_e[b] (cm·h^{-1})
Aug. 20	0.7±0.1	24.2–25.0	8.6–8.9	47.3±3.3
Sept. 9	1.1±0.1	22.1–23.0	8.1–8.9	25.6±1.6
Sept. 21	0.5±0.2	17.5–19.0	8.5	49.0±0.2
Oct. 5	1.3±0.2	15.2–17.0	8.2–8.7	37.2±2.6

[a]Measured at 4 m above lake. Mean ± SD of hourly averages.
[b]Average ± SD.

Because the addition of $HgCl_2$ increased the turbidity of the water, and because suspended particles affect the rate coefficient, some of the difference can be ascribed to this effect. Holley (1977) concluded that K_e increases due to the physical presence of a suspension. The magnitude of the increase depended on the concentration and size of the suspended material.

The K_e values measured with the wind tunnel and the plastic container on the float compare well with values found in the literature. Blain (1969) calculated the fundamental desorption coefficient K_d for ammonia (equivalent to K_e, equation 1) from the results of Stratton (1968). At 20°C and $8.5 < pH < 9.5$, the K_d ranged from 1.46 to 2.07 cm·h^{-1}. No consistent relationship with the temperature could be observed, with K_d at pH 8.5 ranging from 0.7 at 15°C to 2.3 at 30°C. Using data from Wezernak and Gannon (1968) for the loss of nitrogen from the Clinton River and assuming that the total loss was caused by desorption of ammonia, Blain calculated K_d values from 3 to 11 cm·h^{-1} for pH 8 and 20–24°C.

The exchange coefficient, K_e, is a measure of the total resistance to ammonia transfer expressed on a liquid phase basis. It can be expressed in terms of r_l and r_g, the liquid and gas phase resistances for exchange, respectively, as follows (Liss 1973):

$$1/K_e = r_l + r_g. \tag{8}$$

Hicks and Liss (1976) have shown that the transfer of SO_2 from the atmosphere to water is controlled solely by gas phase processes and they expect that "the exchange of other gases of high solubility or rapid aqueous phase reactions (e.g. NH_3, NO_2, SO_3, and HCl) is almost certainly also controlled by resistance in the gas phase." It is therefore likely that ammonia transfer from water to air is also controlled by gas phase resistance. Nevertheless, the measured K_e values can be used to calculate the ammonia loss rate from the water as long as the concentration in the water, the pH, temperature, and wind speed are known.

BLAIN, W. A. 1969. Ammonia nitrogen losses from streams: Discussion. J. San. Eng. Div. Am. Soc. Civ. Eng. 95: 975–978.

CALDER, K. L. 1972. Absorption of ammonia from atmospheric plumes by natural water surfaces. Water, Air, Soil Pollut. 1: 375–380.

EMERSON, K., R. C. RUSSO, R. E. LUND, AND R. V. THURSTON. 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. J. Fish. Res. Board Can. 32: 2379–2383.

HICKS, B. B., AND P. S. LISS. 1976. Transfer of SO_2 and other reactive gases across the air–sea interface. Tellus 28: 348–354.

HOLLEY, E. R. 1977. Oxygen transfer at the air–water interface, p. 117–150. *In* R. J. Gibbs and R. P. Shaw [ed.] Transport processes in lakes and oceans. Plenum Press, New York, N.Y.

HUTCHINSON, G. L., AND F. G. VIETS. 1969. Nitrogen enrichment of surface water by absorption of ammonia volatilized from cattle feedlots. Science 166: 514–515.

JUNGE, C. E. 1963. Air chemistry and radioactivity. Academic Press, Inc., New York, N.Y. xii + 382 p.

LISS, P. S. 1973. Processes of gas exchange across an air–water interface. Deep-Sea Res. 20: 221–238.

PHILBERT, F. J., AND W. J. TRAVERSY. 1973. Methods of sample treatment and analysis of Great Lakes water and precipitation samples. Proc. 16th Conf. Great Lakes Res.: 294–308.

RASMUSSEN, K. H., M. TAHERI, AND R. L. KABEL. 1975. Global emissions and natural processes for removal of gaseous pollutants. Water, Air, Soil Pollut. 4: 33–64.

ROLL, H. U 1965 Physics of the marine atmosphere. Academic Press, Inc., New York, N.Y. viii + 426 p.

STRATTON, F. E. 1968. Ammonia nitrogen losses from streams. J. San. Eng. Div. Am. Soc. Civ. Eng. 94: 1085–1091.

1969. Nitrogen losses from alkaline water impoundments. J. San. Eng. Div. Am. Soc. Civ. Eng. 95: 223–231.

WEZERNAK, C. E., AND J. J. GANNON. 1968. Evaluation of nitrification in streams. J. San. Eng. Div. Am. Soc. Civ. Eng. 94: 883–895.

Chronic Exposure of Trout to Waters with Naturally High Selenium Levels: Effects on Transfer RNA Methylation[1]

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KAISER, I. I., P. A. YOUNG, AND J. D. JOHNSON. 1979. Chronic exposure of trout to waters with naturally high selenium levels: effects on transfer RNA methylation. J. Fish. Res. Board Can. 36: 689–694.

Liver tissues from rainbow (*Salmo gairdneri*) and brown (*S. trutta*) trout chronically exposed to waters with naturally high selenium levels accumulate significant amounts of the element, with values in the 50–70 μg/g range (fresh tissue) not unusual. This accumulation appears to increase with time since older fish from the same waters invariably contain higher liver selenium levels. Studies in other systems suggested that there might be gross alterations in cellular macromolecular methylations in fish chronically exposed to elevated selenium levels. Mature tRNAs isolated from trout livers having both low and high selenium levels, however, showed no differences in their methyl-accepting abilities in a homologous in vitro methylating system. In addition, direct nucleoside analyses on these different tRNA samples showed similar levels of base methylation, with no evidence for methylation abberations. The nucleoside analyses on the tRNAs were similar and in keeping with values reported for other eukaryotic systems.

Key words: selenium, biological methylation, methylation, transfer RNA, nucleoside analysis, *Salmo gairdneri*, *S. trutta*

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Les tissus hépatiques de la truite arc-en-ciel (*Salmo gairdneri*) et de la truite brune (*S. trutta*) exposées de façon chronique à des eaux contenant des concentrations naturelles élevées de sélénium emmagasinent d'importantes quantités de cet élément, des valeurs de 50 à 70 μg/g (tissu frais) n'étant pas rares. Cette accumulation semble augmenter avec le temps car les

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vieux poissons provenant des mêmes eaux ont invariablement de plus fortes concentrations de sélénium dans le foie. Des études effectuées dans d'autres réseaux donnent à penser qu'il puisse se produire des changements grossiers dans la méthylation macromoléculaire cellulaire chez des poissons exposés de façon chronique à des niveaux élevés de sélénium. L'ARNt mûre isolée de foies de truites contenant de bas et de hauts niveaux de sélénium ne montre pas de différences dans son aptitude à accepter le méthyl dans un système de méthylation in vitro homologue. Cependant, les dosages directs de nucléosides sur ces différents échantillons d'ARNt montrent des niveaux semblables de méthylation de base, sans indication de méthylation anormale. Les analyses de nucléosides sur les ARNt sont identiques et s'accordent avec les valeurs signalées pour d'autres systèmes eucaryotiques.

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SELENIUM has the dubious distinction of being classified as both an essential nutrient and environmental pollutant. This dual classification for selenium is due in part to a rather low safety factor of 25–100, which is a measure of the ratio of the toxic dose to the amount needed to prevent selenium deficiency. It is known to be an essential component of several different enzymes, including glycine reductase from *Clostridium sticklandii* (Stadtman 1966; Turner and Stadtman 1973; Cone et al. 1976), formate dehydrogenase from several different bacteria (Pensent 1954, Stadtman 1974), and glutathione peroxidase from several higher species (Noguchi et al. 1973; Oh et al. 1974). It may also function as a detoxification agent of certain heavy metals in animals and promote rapid elimination of other metabolic poisons such as arsenic (Ganther 1974). When administered in toxic amounts, however, selenium can be disruptive to a variety of cellular processes including S-adenosylmethionine (SAM) metabolism (Hoffman 1975, 1977).

In studies on mice, Hoffman (1975, 1977) has shown that acute doses of injected selenite promote a severalfold decrease in liver SAM levels. This decrease results from both an increased utilization of SAM for the production of dimethyl selenide and inactivation of methionine adenosyltransferase, the enzyme responsible for SAM synthesis. The inactivation of methionine adenosyltransferase causes the SAM levels to remain depressed long after cessation of dimethyl selenide synthesis (Hoffman 1977). S-adenosylhomocysteine (SAH), a by-product of biological methylations involving SAM, and an effective inhibitor of tRNA methyl transferases in certain systems (Kerr 1972; Heady and Kerr 1973), increases during selenite injections as might be expected. The lowered SAM levels and elevated SAH concentration in mice treated with selenite suggested that there might be gross alterations in cellular macromolecular methylations in animals chronically exposed to elevated selenium levels. Because of the low safety factor of selenium noted earlier, it is difficult to maintain animals for extended periods chronically exposed to elevated selenium levels without considerable attrition. As such, we decided to examine the methyl-containing nucleosides in tRNAs of trout, chronically exposed to high natural selenium levels in our region and that accumulate significant amounts of liver selenium, for methyl deficiencies. Methyl contents of tRNAs isolated from trout collected in waters in which liver selenium accumulations were both high and low were compared by their ability to accept methyl groups in vitro and by sensitive nucleoside analyses.

Materials and methods — Trout were collected either by rod and reel or obtained through the courtesy of the Wyoming Game and Fish Commission. Their tissues were removed and frozen on dry ice and stored at −70°C until

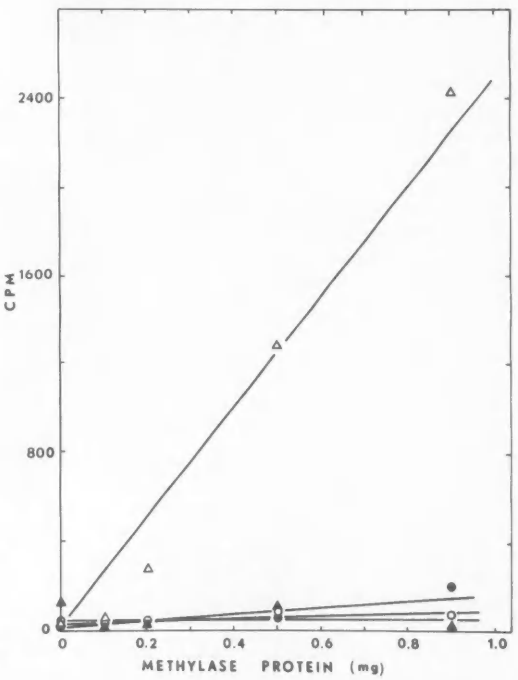

FIG. 1. Methylation of different tRNA samples with increasing concentrations of crude trout-liver methylase. *Escherichia coli* tRNA, △—△; trout liver tRNAs from Pathfinder, ●—●; Twin Buttes, ○—○; and 9-Mile, ▲—▲.

TABLE 1. Selenium levels found in rainbow and brown trout tissues and water samples collected in Wyoming.

Source	County	Date	Species	Selenium concn[d] Tissue (μg/g; wet wt) Muscle	Skin	Liver	Water (μg/L)
Twin Buttes	Albany	Feb. 20/75[a]	Rainbow	3.02[b]		50.3	—
		Apr. 24/75[a]	Rainbow	2.70[b]		52.7	13.3
			Brown	2.36[b]		37.1	13.3
		July 1/75[a]	Brown	1.99[c]	4.51	56.0	12.3
		May 6/76	Rainbow	1.33[c]	—	56.2	—
			Brown	1.06[c]	—	51.4	—
		Apr. 28/77	Brown	—	—	31.7	—
			Brown	—	—	45.4	—
9-Mile	Albany	June 27/75[a]	Rainbow	1.62[c]	4.79[c]	71.4	1.99
			Brown				
		July 3/75[a]	Rainbow				
			(1973 Plant)	1.97[b]		60.0	1.87
			(1974 Plant)	1.68[b]		21.3	1.87
		Apr. 24/77	Rainbow				
			(1975 Plant)	—	—	34.9	—
			Brown				
			(1975 Plant)	—	—	70.6	—
			(1976 Plant)	—	—	17.5	—
Pathfinder	Natrona & Carbon	May 19/76	Rainbow	—	—	6.38	—
			Rainbow	—	—	3.65	—

[a]From T. D. Moore and D. L. Mitchum unpublished data.
[b]Samples were a composite of muscle and skin.
[c]Samples were a composite of like tissue from several fish.
[d]All analyses were carried out by the procedure of Olson 1969.

used. Liver tRNAs were isolated by the phenol method (Kirby 1964) at room temperature and separated from contaminating nucleic acids by column chromatography on Sephadex G-75. Commercial *Escherichia coli* tRNAs were treated as previously described (Young and Kaiser 1975). All tRNA samples had similar electrophoretic patterns when run on 15% polyacrylamide gel slabs (data not shown). Trout liver methylases were prepared from fresh tissues isolated immediately from fish sacrificed in the laboratory on the day of the experiment by the method of Baliga and Borek (1974), except that only 2 vol of buffer were used for the initial homogenization and a Tekmar homogenizer replaced the Sorvall Omni-Mixer.

Assays for tRNA methyl-accepting activity were carried out in duplicate as described below, using 25 μg tRNA (0.5 A_{260}-units) and the indicated amount of crude methylase. Controls containing no added tRNAs were run at each methylase concentration to correct for background. Following 30-min incubation at 37°C each reaction mixture (0.2 mL) was precipitated by the addition of 5 mL cold 7% TCA in the presence of 0.5 mg BSA. The precipitates were washed 3 times with 5-mL portions of cold 7% TCA by repeated resuspension and pelleting by centrifugation. The final pellet was resuspended in 3 mL Biofluor (New England Nuclear), added to Beckman polypropylene Bio-vials, and counted in a liquid scintillation counter at about 90% efficiency. The [^{14}C-methyl]-S-adenosylmethionine (International Chemical and Nuclear Corp.) had a specific activity of 1.92GBq/mmol (51.8 mCi/mmol).

Base composition analyses of the tRNAs were done using the chemical tritium-labeling method (Randerath et al. 1972; Randerath et al. 1974). The identity of all radioactive spots was verified by parallel analyses of commercial nucleoside preparations except for 3-(3-amino-3-carboxypropyl)-uridine which is tentatively identified by its position relative to the map of Randerath et al. (1974).

Results and discussion — Earlier work indicated that liver tissue from fish chronically exposed to waters with high selenium levels accumulate significant amounts of the element (T. D. Moore and D. L. Mitchum unpublished data). Values in the 50–70-μg/g range (fresh tissue) are not unusual. Muscle tissue is comparatively low in the element, which is in keeping with selenium concentrations measured in wild trout collected from waters of New York State. In this work, selenium levels ranged from 0.5 to 1 μg/g in dried tissue from decapitated and eviscerated fish (Pakkala et al. 1972). Areas of the northeastern part of the United States are generally considered low in selenium (Rosenfeld and Beath 1964) and would not be expected to accumulate significant amounts of selenium, particularly

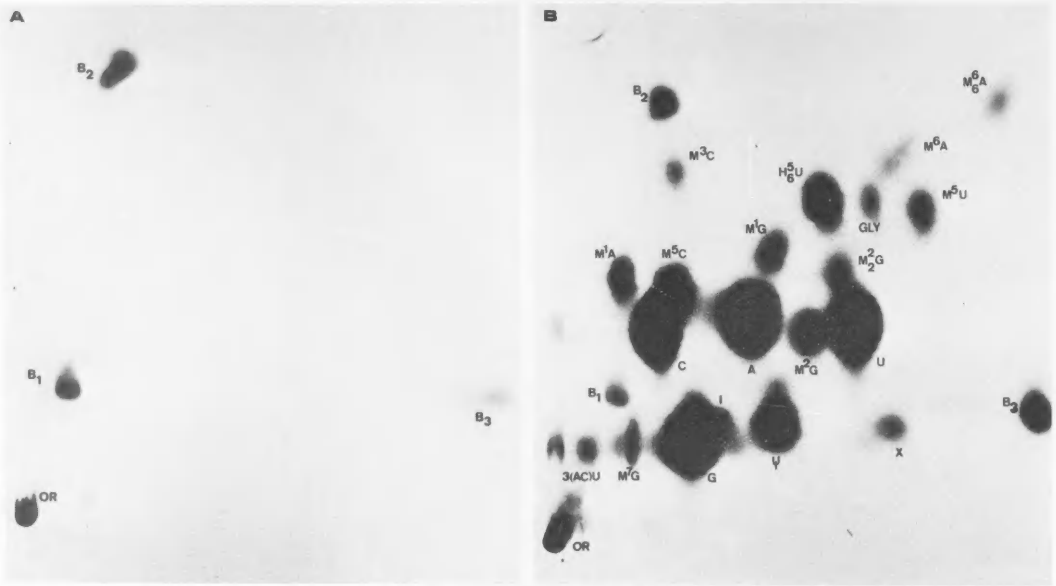

FIG. 2. (A) Map of ^{3}H-radioactivity from a reaction mixture lacking RNA. (B) Map of ^{3}H-radioactivity obtained from the labeling of 3.6 nmol of nucleosides from 9-Mile Reservoir brown trout tRNA. Fluorographic exposure of the X-ray film was for 62 h at −75°C. OR (origin), B_1, B_2, and B_3 contain unidentified material present in reaction mixtures lacking RNA. The following represent 2′,3′-[^{3}H]-trialcohol derivatives of the nucleoside; *3(AC)U = 3-(3-amino-3-carboxypropyl) uridine, **M^7G = 7-methylguanosine, G = guanosine, I = nosine, ψ = pseudouridine, **M^1A = 1-methyladenosine, C = cytidine, M^5C = methylcytidine, A = adenosine, U = uridine, M^1G = 1-methylguanosine, M^2G = 2-methylguanosine, M_2^2G = 2,2-dimethylguanosine, **M^3C = 3-methylcytidine, H_6^5U = 5,6-dihydrouridine, M^5U = 5-methyluridine, M^6A = 6-methyladenosine, M_6^6A = 6,6-dimethyladenosine, × = unidentified, GLY = glycerol derived from the breakdown of alkali labile nucleosides. *Identified only by comparison with the map of Randerath et al. 1974. **Not corrected for partial loss due to alkaline degradation (Randerath et al. 1972; Johnson and Horowitz 1971).

in muscle and skin tissues. The results in Table 1 further suggest that at least in liver tissues, selenium appears to accumulate with time since older fish from the same waters invariably contain higher selenium levels. Other studies, where the entire fish was assayed for selenium content, indicated that there was an equilibrium between the fish and its surroundings (Nielsen and Gissel–Nielsen 1978). This may reflect different forms of selenium in different tissues, some of which are readily exchanged and some which have a much longer biological half-life.

The availability of fish with elevated levels of selenium in liver tissues provided an opportunity to examine macromolecules isolated from these tissues for possible methyl deficiencies. Transfer RNAs were isolated from trout collected in waters in which liver selenium accumulations were both high and low (Table 1). The methyl-accepting ability of these preparations was then compared in a homologous in vitro methylation system using a crude preparation of tRNA methylases. There was no apparent difference in the methyl-accepting ability of any of the trout liver tRNAs (Fig. 1), indicating that high selenium levels do not promote the formation of undermethylated, mature tRNAs in selenium-rich trout liver. As expected *E. coli* tRNAs readily served as methyl acceptors in this in vitro system.

Friedman (1977) recently reported that the in vitro methylation assay may not give an accurate reflection of the methyl deficiency of tRNA. Therefore, we determined directly the methylated nucleosides present in trout samples by the tritium-labeling method of Randerath et al. (1972). Figure 2 shows a representative fluorogram from a two-dimensional cellulose thin-layer chromatogram of the tritium-labeled nucleoside trialcohol mixture obtained from trout. Nucleoside compositions from the tRNAs of rainbow trout from Twin Buttes, 9-Mile, and Pathfinder reservoirs all showed similar levels of base methylation (Table 2). Since we were interested in comparative data, no corrections were made for partial losses of 7-methylguanosine, 1-methyladenosine, and 3-methylcytidine that occur during the tritium-labeling procedure. The sum of the methylated constituents in tRNAs from the low selenium sample, 6.21 mol % (Pathfinder rainbow trout) was in the same range, 5.67–7.03 mol %, as

TABLE 2. Comparison of nucleoside compositions of tRNAs from liver of fish containing low and high levels of selenium. The average of duplicate determinations are reported for each sample. See legend to Fig. 2 for explanation of abbreviations. ND = not determined, NP = not present in detectable amounts.

	Mol %					
	Rainbows			Browns		
Nucleoside	Twin Buttes	9-Mile	Pathfinder	Twin Buttes	9-Mile	Carp[a]
G	27.93	28.53	28.56	27.67	28.06	30.10
A	17.53	17.24	17.43	18.90	19.07	19.00
C	26.86	28.68	26.71	26.67	26.72	29.20[c]
U	15.20	14.85	15.49	15.59	15.41	17.60[d]
M^1G	0.58	0.33	0.77	0.70	0.65	0.58
M^2G	0.75	0.80	1.15	0.97	1.04	
M_2^2G	0.51	0.49	0.53	0.49	0.39	0.60
M^7G	1.02	0.74	0.42	0.43	0.39	ND
M^1A	0.62	0.66	0.86	0.64	0.73	ND
M^6A	0.12	0.13	0.10	0.33	0.18	1.04[b]
M_6^6A	0.56	0.27	0.29	0.12	0.18	<0.02
M^3C	0.23	0.24	0.22	0.19	0.18	ND
M^5C	1.95	1.27	1.27	1.43	1.24	(Included in C)
3(AC)U	0.63	ND	0.38	0.28	0.26	ND
M^5U	0.69	0.74	0.60	0.42	0.67	(Included in U)
H_6^5U	1.36	0.64	1.84	1.52	1.09	ND
Ψ	2.96	3.64	2.83	3.08	3.26	4.35
I	0.60	0.94	0.32	0.49	0.27	ND
X	NP	NP	0.35	NP	0.31	ND
Σ Methylated constituents	7.03	5.67	6.21	5.72	5.65	—

[a]Zaitseva et al. (1962).
[b]Probably includes M^1A.
[c]Includes M^5C.
[d]Includes M^5U.

tRNAs from the high selenium samples (Twin Buttes and 9-Mile). There was also no indication that brown trout from the same waters produce undermethylated, mature tRNAs (Table 2). For comparative purposes, we have included the partial nucleoside composition of carp (*Cyprinus carpio*) liver. This composition was reported over 15 yr ago (Zaitseva et al. 1962), but still reflects *the only* published nucleoside analysis for fish tRNAs that we could find.

We have also examined the nucleoside composition of tRNAs from a selenium accumulator plant *Astragalus bisulcatus* (J. D. Johnson, P. A. Young, and I. I. Kaiser, University of Wyoming, unpublished data). Accumulations of up to 10 000 μg/g have been reported for this species (Rosenfeld and Beath 1964). When compared with other plant tRNA samples (wheat germ and *Saccharomyces cerevisiae*), the methylated base contents show no apparent changes. Thus, chronic exposure of trout and selenium accumulator plants to naturally high selenium levels does not affect methylation levels in mature tRNAs. Whether other cellular methylations are affected by this exposure is presently unknown.

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BALIGA, S., AND E. BOREK. 1974. An early effect of estrogen on the tRNA-methyltransferases of rat uterus. Endocrinology 94: 815–821.

CONE, J. E., R. MARTIN DEL RIO, J. N. DAVIS, AND T. C. STADTMAN. 1976. Chemical characterization of the selenoprotein component of clostridial glycine reductase: Identification of selenocysteine as the organoselenium moiety. Proc. Natl. Acad. Sci. USA 73: 2659–2663.

FRIEDMAN, S. 1977. Alterations in tRNA modification in mammalian systems: The effect of ethionine. Nucleic Acids Res. 4: 1853–1871.

GANTHER, H. E. 1974. Biochemistry of selenium, p. 546–614. *In* Selenium. Van Nostrand Reinhold Co., New York, N.Y.

HEADY, J. E., AND S. J. KERR. 1973. Purification and characterization of glycine N-methyltransferase. J. Biol. Chem. 248: 69–72.

HOFFMAN, J. L. 1975. Selenium toxicity and methyl group metabolism. Fed. Proc. 34: 924.

1977. Selenium toxicity, depletion of liver S-adenosylmethionine, and inactivation of methionine adenosyltransferase. Arch. Biochem. Biophys 179: 136–140

JOHNSON, J. D., AND J. HOROWITZ. 1971. Characterization of ribosomes and RNAs from *Mycoplasma hominis*. Biochim. Biophys. Acta 247: 262–279.

KERR, S. J. 1972. Competing methyltransferase systems. J. Biol. Chem. 247: 4248–4252.

KIRBY, K. S. 1964. Isolation and fractionation of nucleic acids. Prog. Nucleic Acid Res. Mol. Biol. 3: 1–31.

NIELSEN, M. G., AND G. GISSEL-NIELSEN. 1978. Sensitivity of trout to chronic and acute exposure to selenium. Agric. Environ. 4: 85–91.

NOGUCHI, T., A. H. CANTOR, AND M. L. SCOTT. 1973. Mode of action of selenium and vitamin E in prevention of exudative diathesis in chicks. J. Nutr. 103: 1502–1511.

OH, S. H., H. E. GANTHER, AND W. G. HOEKSTRA. 1974. Selenium as a component of glutathione peroxidase isolated from ovine erythrocytes. Biochemistry 13: 1825–1829.

OLSON, O. E. 1969. Fluorometric analysis of selenium in plants. J. Assoc. Off. Anal. Chem. 52: 627–634.

PAKKALA, I. S., W. H. GUTENMANN, D. J. LISK, G. E. BURDICK, AND E. J. HARRIS. 1972. A survey of the selenium content of fish from 49 New York State waters. Pestic. Monit. J. 6: 107–114.

PENSENT, J. 1954. The need for selenite and molybdate in the formation of formic dehydrogenase by members of the *Coli-aerogenes* group of bacteria. Biochem. J. 57: 10–16.

RANDERATH, E., C.-T. YU, AND K. RANDERATH. 1972. Base analysis of ribopolynucleotides by chemical tritium labeling. Anal. Biochem. 48: 172–198.

RANDERATH, K., E. RANDERATH, L. S. Y. CHIA, AND B. J. NOWAK. 1974. Base analysis of ribopolynucleotides by chemical tritium labeling: An improved mapping procedure for nucleoside trialcohols. Anal. Biochem. 59: 263–271.

ROSENFELD, I., AND O. A. BEATH. 1964. Selenium. Academic Press, New York, N.Y. 411 p.

STADTMAN, T. C. 1966. Glycine reduction to acetate and ammonia. Arch. Biochem. Biophys. 113: 9–19.

1974. Selenium biochemistry. Science 183: 915–922.

TURNER, D. C., AND T. C. STADTMAN. 1973. Purification of protein components of the clostridial glycine reductase system and characterization of protein A as a seleno-protein. Arch. Biochem. Biophys. 154: 366–381.

YOUNG, P. A., AND I. I. KAISER. 1975. Aminoacylation of *Escherichia coli* cysteine tRNA by selenocysteine. Arch. Biochem. Biophys. 171: 483–489.

ZAITSEVA, G. N., T. M. DMITRIVA, H. CH'ANG-FA, AND A. N. BELOYERSKII. 1962. A comparative study of the nucleotide composition of soluble ribonucleic acids from some species of bacteria and animals. Dokl. Akad. Nauk SSSR 147: 1211–1214.

Density Estimates of *Mysis relicta* in Lake Michigan[1]

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GROSSNICKLE, N. E., AND M. D. MORGAN. 1979. Density estimates of *Mysis relicta* in Lake Michigan. J. Fish. Res. Board Can. 36: 694–698.

Density estimates of *Mysis relicta* based on night vertical net tows at stations of 30–50 m in Lake Michigan were about an order of magnitude larger than previous estimates based on sled tows. Significantly greater density estimates were obtained in night vertical net tows than those collected before sunset at these shallow stations. However, density estimates based on sled tows during daylight were not significantly different from those based on night vertical net tows at a 115-m station in Lake Michigan. At this deep station, a substantially larger percentage of late instar mysids was collected in sled tows than in night vertical net tows.

Key words: Mysis relicta, Lake Michigan, density estimates, vertical net tows, epibenthic sled tows

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Les estimations de densité de *Mysis relicta* fondées sur des prises verticales nocturnes au filet à des stations de 30 m à 50 m dans le lac Michigan sont supérieures d'environ un ordre de grandeur à des estimations antérieures fondées sur des prises au traîneau. A ces stations peu profondes, on obtient des estimations de densité nettement plus élevées dans les prises verticales nocturnes au filet que dans celles effectuées avant le coucher du soleil. Cependant, les estimations de densité fondées sur des prises diurnes au traîneau ne diffèrent pas de façon significative de celles fondées sur des prises verticales nocturnes au filet à une station de 115 m dans le lac Michigan. A cette station profonde, on a recueilli un pourcentage nettement plus élevé d'instars de mysides avancés dans des prises au traîneau que dans des prises verticales nocturnes au filet.

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SAMPLING methods for estimating population densities of *Mysis relicta* must accommodate the diel changes in its vertical distribution. Beeton (1960) concluded that light is the primary environmental factor controlling the vertical migration of *Mysis*, although rapid temperature changes were also found to limit the upper extent of migration. During the day, he seldom found mysids more than 1.8 m above the bottom at depths of 91 m or less in Lake Michigan. In deeper regions (i.e. 152 m), Beeton collected mysids as much as 7 m above the bottom during daylight. Similarly, Morgan et al. (1978) reported *Mysis* to be almost entirely benthic to depths as great as 200 m in the transparent waters of Lake Tahoe and almost entirely pelagic during the day at depths >400 m.

Investigators have generally estimated mysid densities in the Laurentian Great Lakes by sampling during daylight with a net mounted horizontally on an epibenthic sled (McWilliam 1970; Reynolds and DeGraeve 1972; Morgan and Beeton 1978) or in vertical net tows (Carpenter et al. 1974). However, the ability of these methods to provide accurate density estimates varies considerably with light conditions which in turn vary with depth and the time of sampling.

The objectives of this study are to compare year-long day vs. night density estimates of *Mysis* for a nearshore region (i.e. 50 m or less) of Lake Michigan based on vertical net tows, to compare these with previously published day estimates from an epibenthic sled at similar depths in Lake Michigan (Reynolds and DeGraeve 1972), and to compare mysid density estimates from night vertical net tows and day epibenthic sled tows at a midwater (115-m) station. This direct comparison of night vertical net tows with day sled tows also provides a test of the capacity of each method to sample all portions of the mysid population efficiently.

Materials and methods — Day vs. night tests — Vertical tows were taken with a 0.75-m diam, 0.57-mm (no. 0 mesh) plankton net from the bottom to the surface at 0.33 $m \cdot s^{-1}$. About 2–3 m of line was added after the weighted cod end of the net touched the bottom. This allowed the towing ring to touch the bottom. Density could be estimated as the mysids above 1 m^2 of lake bottom because vertical net tows sampled the entire water column. Except on July 2, 1975 all vertical net tows were taken either before sunset (i.e. "day") or at least 1 h after sunset (i.e. "night"). Regular sampling occurred at ~2–3-wk intervals from July 1975 to May 1976 at 30-, 40-, and 50-m stations off Milwaukee. At least three replicates were taken at each station. Samples were preserved in 10% formalin, and all *Mysis* were counted.

Tests of sampling gear — Epibenthic sled sampling was compared with vertical net tows on April 22, May 27, and July 13, 1976 at a 115-m station ~30 km northeast of Milwaukee. Six vertical net tows were taken at night using the methods described above. Two or three sled tows were made with a 1-m^2, 0.57-mm (no. 0 mesh) horizontally mounted net fitted with a net release and towed at an average speed of 1.17 $m \cdot s^{-1}$ (Morgan and Beeton 1978). In the laboratory, mysids in sled tow samples were counted and classified as one of five instars on the basis of statolith diameter (Morgan and Beeton 1978) after subsampling with a Folsom Plankton Splitter. Density estimates for vertical net tows and sled tows were converted to mysids per square metre.

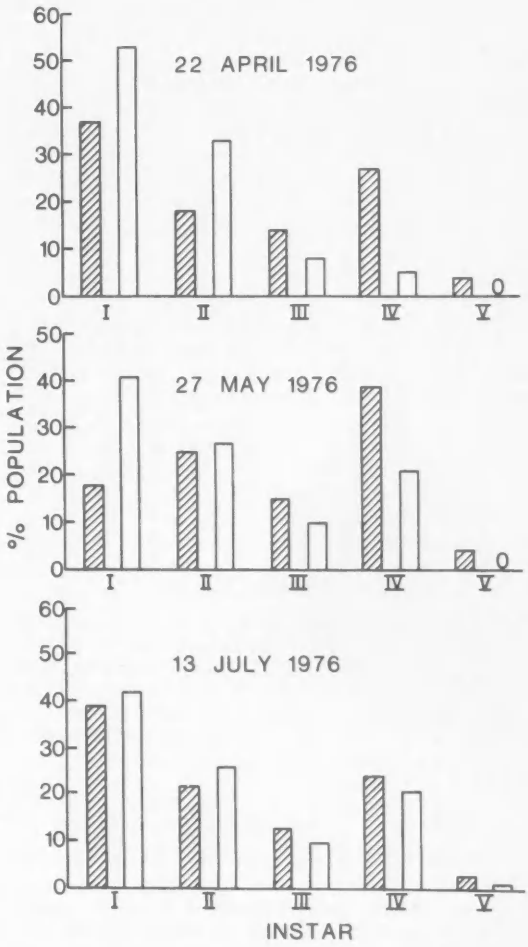

FIG. 1. Histogram illustrating the proportion of each instar present in day sled tows and night vertical net tows. Hatched bars indicate sled tows and open bars indicate vertical net tows.

Results and discussion — Tests of sampling gear — Mysid density at a midwater (115-m) station was estimated by sled tows during daylight and vertical net tows taken at night. In April more mysids were collected in the vertical net tows than the sled tows, while in May the opposite was true. However, sample variances were

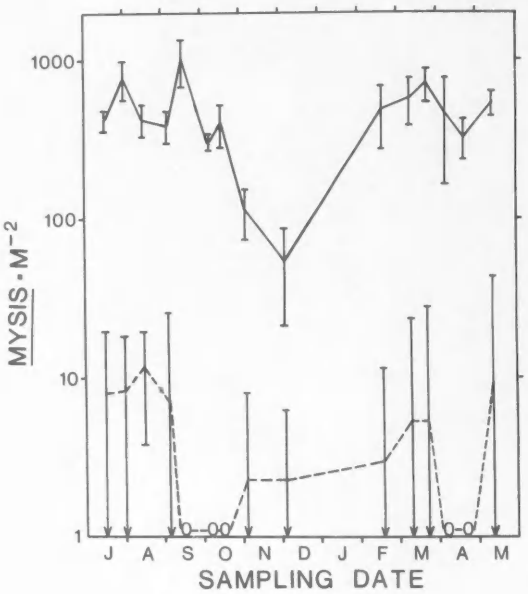

FIG. 2. Density estimates of *Mysis relicta* based on day (dashed line) and night (solid line) vertical net tows at the 30–50-m stations in Lake Michigan. Vertical bars indicate 95% confidence intervals. Arrows indicate values <1. Zero indicates that all replicate tows during daylight were zero on that date. Solid or dashed lines connect mean values.

too large to make the differences statistically significant ($P < 0.05$, *t*-test) on both sampling dates. In July the density estimates of the two methods were similar.

Mysid density estimates and mean number of *Mysis* per square metre ± SD (n) based on night vertical net tows (VT) and day sled tows (Sled) at a 115-m station in Lake Michigan:

	Sled	VT
Apr. 22, 1976	170 ± 34(3)	553 ± 244(6)
May 27, 1976	333 ± 78(3)	256 ± 76(6)
July 13, 1976	293 ± 22(2)	320 ± 39(6)

An analysis of the mysid population structure obtained from the vertical net tows and sled tows on these dates indicated that these methods were not sampling all instars equally (Fig. 1). On April 22 and May 27, mysids of the third, fourth, and fifth instar made up a significantly larger percentage of the catches from the sled tows than the vertical net tows while first and second instar individuals were more abundant in vertical net tows ($P < 0.005$). The same trend was observed on July 13, but was not significant ($P < 0.5$). It is not possible at this time to demonstrate whether the sled tows were selecting against smaller forms or the vertical net tows were selecting against the larger forms, but the large percentage of small individuals and the nearly complete absence of fifth instar individuals in the vertical tows suggest the latter possibility.

The apparent bias of vertical net tows against large individuals in perplexing. A large portion of the fifth instar mysids apparently either remain on the bottom during the night or suffer very high rates of mortality during their vertical migration. J. Janssen (unpublished data) found that large mysids, especially those corresponding to our Instar V, dominated the stomachs of large alewives in this region of Lake Michigan. The large mysids that survive fish predation may therefore be those that limit their vertical migrations. This may account for the presence of large mysids in horizontal epibenthic sled tows and their paucity in night vertical net tows.

Day vs. night tests — On all sampling dates, night vertical tows provided significantly greater density estimates than tows during daylight ($P < 0.01$, *t*-test) at the 30–50-m stations (Fig. 2). The difference between night and day estimates was generally one to two orders of magnitude. However, numerous downwelling events occurred along the Wisconsin shore of Lake Michigan during September and October 1975 that resulted in isothermal conditions above 10°C at the 30-m station (Bowers 1977). Few if any mysids were collected during night tows at the 30-m station during September and October. Thus, all density estimates in Fig. 2 were based on mysid samples collected at stations of 30–50 m depth except those samples collected during September and October, when only counts from the 50-m station were used.

A direct relationship between mysid density and light intensity is characterized in Fig. 3. Samples collected at the 50-m station shortly before, during, and after sunset contained consistently more *Mysis* as time progressed. The last sample, taken about 20 min after sunset, was similar in abundance to the night samples at this station.

The year-long nighttime density estimates presented in Fig. 2 illustrate the ability of vertical net tows to provide highly replicable population estimates. These estimates ranged from a low of $54.1 \cdot m^{-2}$ in December to a high of $1019 \cdot m^{-2}$ in September and averaged 466. Over the entire year, the density estimates remained remarkably constant except for nearly an order of magnitude decline from October to December. By February, however, densities had returned to previous levels. The October–December decline appeared much too rapid to be solely due to mortality and the subsequent increase in the population occurred too early to be due to recruitment (Reynolds and DeGraeve 1972; Morgan and Beeton 1978). Thus horizontal migration, either onshore or offshore, seems a plausible explanation of the seasonal fluctuations.

Night vertical net tows at 30–50-m stations in Lake Michigan contained much greater mysid densities than vertical net tows taken at similar depths in three other

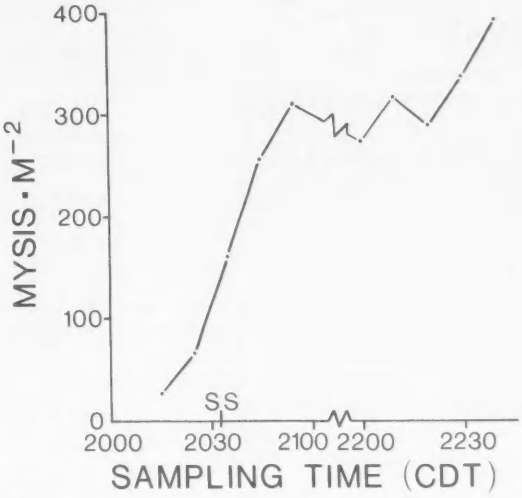

FIG. 3. Density of *M. relicta* taken in vertical net tows before, during, and after sunset (SS) at a 50-m station in Lake Michigan. Densities are based on single vertical net tows. Times reported are at the midpoint of the tow.

Laurentian Great Lakes by Carpenter et al. (1974). They reported densities of 13.00, 5.75, and 24.28 mysids·m^{-2} in the 25–50-m depth zone of Lakes Huron, Ontario, and Superior, respectively. Carpenter et al. sampled during the day and at night and reported that no variation in their density estimates was attributable to differences in time of sampling. In contrast, our density estimates from day and night vertical net tows were significantly different ($P < 0.01$). These differing conclusions may be partially due to differences in location of sampling stations and replication. Carpenter et al. collected one vertical net haul from stations representing all regions of each lake whereas we collected one to five replicate vertical hauls both before and after sunset from stations located within 5 km of each other. Consequently, variability among samples and large-scale horizontal patchiness may have been a greater factor in their statistical analysis than ours. These factors may have combined to mask the effect of daylight on their density estimates.

It is possible that the differences in density estimates of mysids from Lake Michigan and Lakes Huron, Ontario, and Superior reflect true differences in the size of the respective populations. This seems unlikely, however, as density estimates from deeper portions of Lake Michigan (McWilliam 1970; Morgan and Beeton 1978) are very similar to those reported by Carpenter et al. for deeper portions of the lakes they studied.

Mysid density estimates based on day sled net tows by Reynolds and DeGraeve (1972) for seven stations ranging in depth from 18 to 73 m in southeastern Lake Michigan were also much lower than our density estimates. They reported a mean density of 14.3 mysids·m^{-2} over their 1-yr study, with monthly estimates ranging from a low of 0.3 mysids·m^{-2} in December to 64.2 mysids·m^{-2} in August.

Why are mysid density estimates based on daylight vertical net tows so much lower than those based on night vertical net tows at depths of 30–50 m in Lake Michigan? One possibility is that the mysids can see and avoid an approaching net, towline, and bridle at these depths. Fleminger and Clutter (1965) collected many more mysids in oblique tows through an experimental pool in darkness than they did in full or reduced light. They postulated that visual perception of sampling gear may have accounted for these differences. Beeton (1960) found sufficient light for *Mysis* to detect foreign objects at depths up to 100 m even during the spring phytoplankton bloom in Lake Michigan. Thus, visual perception of sampling gear may account for our low density estimates from vertical net tows taken during daylight.

It is also possible that the mysids were too close to the bottom to be effectively sampled by day vertical net and sled tows taken at shallow or intermediate depths. Evidence for this hypothesis is provided by our density estimates at the 115-m station. Here, day sled tows and night vertical net tows provided similar population estimates, while day vertical tows yielded no animals (Morgan and Beeton 1978). These data suggest that at this depth, mysids were slightly off the bottom (in order for the sled to catch them) but still close enough to the bottom so that vertical net tows were ineffective. Thus, proximity to the bottom seems to be the governing characteristic that determines which sampling method is most effective and, as previously stated, light is the primary factor that determines the proximity of the mysids to the bottom.

Therefore, since mysids are planktonic during the night (Beeton 1960), night vertical net tows are always an effective means of sampling the population. During the day, mysids are also found to be mainly planktonic in very deep water (Robertson et al. 1968; Morgan et al. 1978) and so day vertical net tows may be effective at these depths. Day sled tows, on the other hand, would only be effective at intermediate depths (such as 115 m in this study) where the mysids are just off the bottom due to low light intensity.

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BEETON, A. M. 1959. Photoreception in the opossum shrimp *Mysis relicta* Lovén. Biol. Bull. Woods Hole, Mass. 116: 204–216.

1960. The vertical migration of *Mysis relicta* in Lakes Huron and Michigan. J. Fish. Res. Board Can. 17: 517–539.

BOWERS, J. A. 1977. The feeding habits of *Diaptomus ashlandi* and *Diaptomus sicilis* in Lake Michigan and the seasonal vertical distribution of chlorophyll at a nearshore station. Ph.D. Thesis, Univ. Wisconsin-Madison, Madison, Wis. 138 p.

CARPENTER, G. F., E. L. MANSEY, AND N. H. F. WATSON. 1974. Abundance and life history of *Mysis relicta* in the St. Lawrence Great Lakes. J. Fish. Res. Board Can. 31: 319–325.

FLEMINGER, A., AND R. I. CLUTTER. 1965. Avoidance of towed nets by zooplankton. Limnol. Oceanogr. 19(1): 96–104.

MCWILLIAM, P. S. 1970. Seasonal changes in abundance and reproduction in the opossum shrimp *Mysis relicta* Lovén in Lake Michigan. M.S. Thesis. Univ. Sydney, Sydney, Australia. 94 p.

MORGAN, M. D., AND A. M. BEETON. 1978. Life history and abundance of *Mysis relicta* in Lake Michigan. J. Fish. Res. Board Can. 35: 1165–1170.

MORGAN, M. D., S. T. THRELKELD, AND C. R. GOLDMAN. 1978. Impact of the introduction of kokanee (*Oncorhynchus nerka*) and opossum shrimp (*Mysis relicta*) on a subalpine lake. J. Fish. Res. Board Can. 35: 1572–1579.

REYNOLDS, J. B., AND G. M. DEGRAEVE. 1972. Seasonal population characteristics of the opossum shrimp, *Mysis relicta*, in southeastern Lake Michigan, 1970–1971. Proc. Conf. Great Lakes Res. (Int. Assoc. Great Lakes Res.) 15: 117–131.

ROBERTSON, A., C. F. POWERS, AND R. ANDERSON. 1968. Direct observations of *Mysis relicta* from a submarine. Limnol. Oceanogr. 13: 700–702.

PERSPECTIVES

Bioeconomics of Commercial Fisheries Management[1]

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MITCHELL, C. L. 1979. Bioeconomics of commercial fisheries management. J. Fish. Res. Board Can. 36: 699–704.

Canada has indicated that its management of fisheries is based on the "best-use" principle. This principle as an operational concept can be defined as the maximization of socioeconomic returns subject to both biological and economic constraints. This paper examined how this principle can become operational in a management sense by defining the similarities and differences in the approach to fisheries management by biologists and economists and by indicating how these roles can be combined for this management.

Key words: fisheries, management, biology, economics, bioeconomics

MITCHELL, C. L. 1979. Bioeconomics of commercial fisheries management. J. Fish. Res. Board Can. 36: 699–704.

Le Canada a indiqué que sa gestion des pêches est fondée sur le principe de la «meilleure utilisation». Comme concept opérationnel, ce principe peut être défini comme étant le fait de porter au maximum les bénéfices socio-économiques sujets aux contraintes à la fois biologiques et économiques. Nous examinons dans le présent article la manière dont ce principe peut devenir opérationnel au sens de la gestion. A cette fin, nous définissons les ressemblances et les différences dans l'approche à la gestion des pêches adoptée par les biologistes et les économistes, et nous indiquons comment ces rôles peuvent être combinés.

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COMMERCIAL fisheries resource management has been so dominated by biological considerations that it is widely regarded as a biological discipline (Watt 1968). It is only in relatively recent years that economics has begun to play an increasingly important role in this management. This is due mainly to the realization that fisheries exploitation, as indeed the exploitation of all resources, occurs for economic reasons. In almost every other activity economics, concerned with the maximization of returns brought about by the best allocation of the factors of production, i.e. resources, labor, and capital, has been the dominant management discipline. This paper is not concerned with dominance but with how biology and economics complement each other in fisheries management and how this can be used to improve management.

Commercial Fisheries Management: Major Objectives

Commercial fisheries management means different things to fishermen, industry, and to those charged with the management and administration of fisheries. After over 100 years of federal government responsibility for fisheries in Canada, it is only recently that the Department of Fisheries and Oceans, the agency concerned, has enunciated objectives and goals for fisheries management (Environment Canada 1976). The principle governing such management is that of "best use" defined as "the sum of net social benefits (personal income, occupational opportunity, consumer satisfaction and so on) derived from the fisheries and the industries linked to them" (Environment Canada 1976, p. 53).

"Best use" indicates that the primary purpose of fisheries management is to serve man and society. The present challenge is, however, to make this principle an operational concept and this implies a combination of biological and socioeconomic factors and considerations in fisheries management. To understand how this might be accomplished it is necessary to look at the tasks to be undertaken, identify the respective roles of the biologist and social scientist, and indicate how these roles can be integrated into an effective management framework. These can be facilitated by means of a simple management model (Fig. 1).

Although the model disguises the scope of management complexities, clearly the exercise of fisheries

[1]This is the revised text of a paper presented to the Joint Meeting of the 1978 cold-water workshop and the American International Chapter of the American Fisheries Society held in Fredericton, N.B., October 4–6, 1978.

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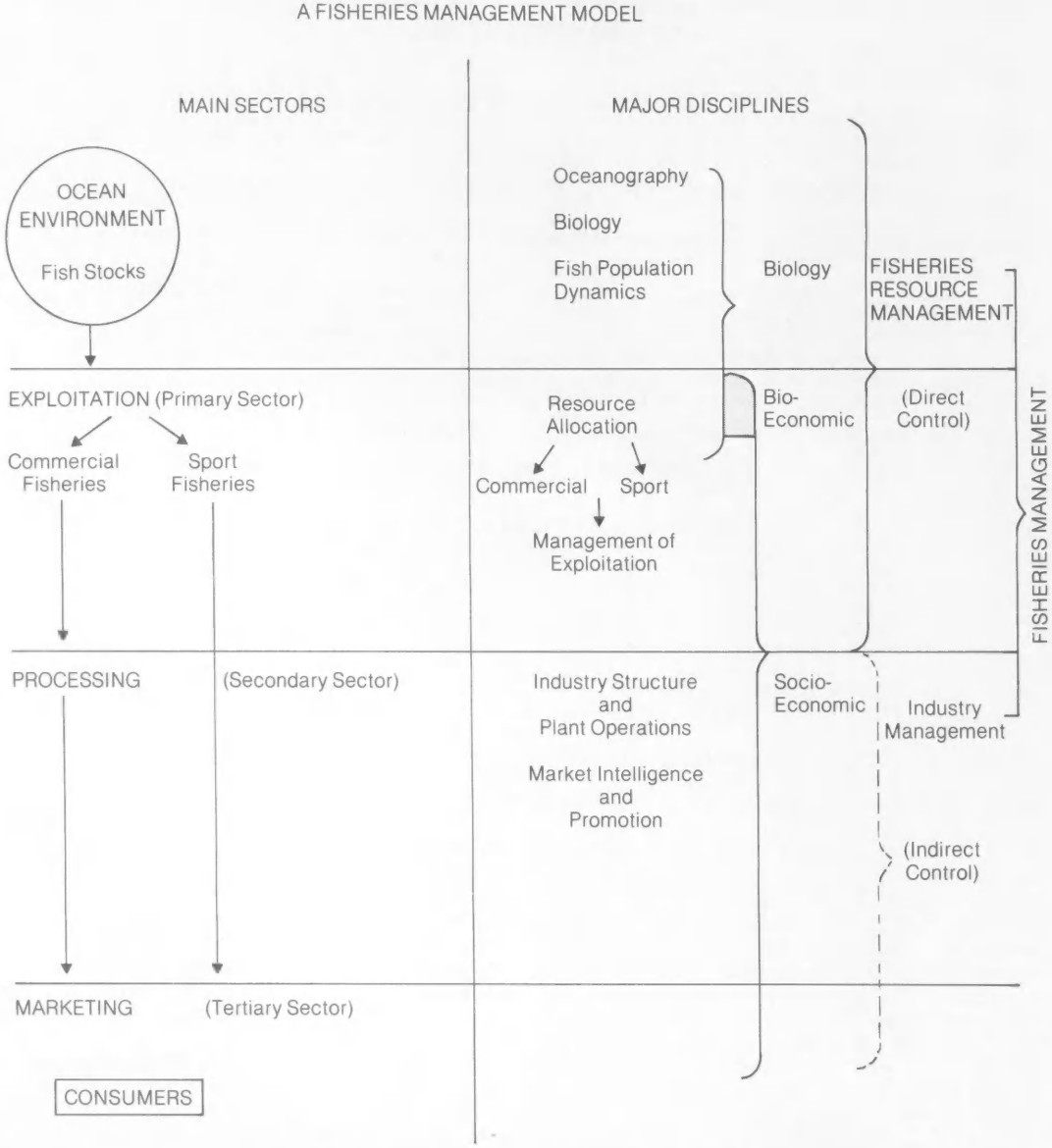

FIG. 1. A fisheries management model indicating the major disciplines — either biological or socioeconomic — that are involved in fisheries management by major sectors, i.e. the primary fishing sector, the secondary manufacturing or processing sector, and the tertiary or marketing sector.

management requires an awareness of the problems of the industry including not only the primary fishing sector but also the secondary manufacturing or processing sector and the tertiary market sector (the consumers), since all these affect each other. Management problems may therefore emanate from either direction, that is, from the resource state or from market conditions (e.g. drop in demand for fish products) or may be caused by structural defects in either or both primary and secondary sectors of the industry. As a result, fisheries management is an extremely complex subject, so complex that it is thought by those in-

volved in it to be a completely new challenge for management sciences (T. D. Iles, unpublished data).

Concentrating on fisheries resource management, the model makes it easy to demarcate the contribution of biology and economics to this management and where these two disciplines interact. The biologist's major concerns are with research on the environment, fish population dynamics, man's impact on these populations, etc., to indicate what levels of exploitation can be sustained without jeopardizing the stocks. The job of establishing quotas or total allowable catches (TAC's), and particularly in the allocation of these, whether to the commercial and sport fisheries or between various fleet segments, requires interaction with the economist. These are the areas where economics as a discipline comes into its own because it is designed to tackle problems caused by competing uses for the resources, and to bring about, as stated earlier, the best allocation of the factors of production to maximize benefits to society as a whole. In fact, this becomes a problem for which optimization techniques can be used (Clark 1971; Sancho and Mitchell 1975). The problem is thus to maximize socioeconomic returns subject to both biological (the resource) and economic constraints (labor and capital). This is essentially what is required to ensure the "best use" of fisheries resources.

Now that the respective roles for the biologist and economist in commercial fisheries management have been identified in general terms, more specific comments can be made about the complementarity of biology and economics in this management.

The Economic Theory of Fisheries

The complementarity of biology and economics in fisheries is due to the fact that the economic theory of fisheries is based on biological foundations, namely the biological production function of fish population growth and especially man's impact, through his fishing effort, on this growth. Being largely empirical, the economic theory developed is based on the pattern of behavior displayed in the exploitation of the resource. Economists studied this exploitation and then generalized it through a theoretical model which purported to resemble reality closely. Because economists for a long time thought that fisheries exploitation was no different from that of any other natural resource, economic theories of fisheries were late in developing. These evolved from Gordon's seminal article (1953) which discovered that fisheries had a few quirks of its own due to biological behavior and its common property nature. Today fisheries economics is a special area of study in its own right and interesting developments are taking place that have important implications for resource management.

The biological foundation of the economics of fisheries is the source of both similarities and differences between the two disciplines and their approaches to fisheries resource management. The standard biological model, used as the starting point in current economic theory, is the logistic model of population growth ap-

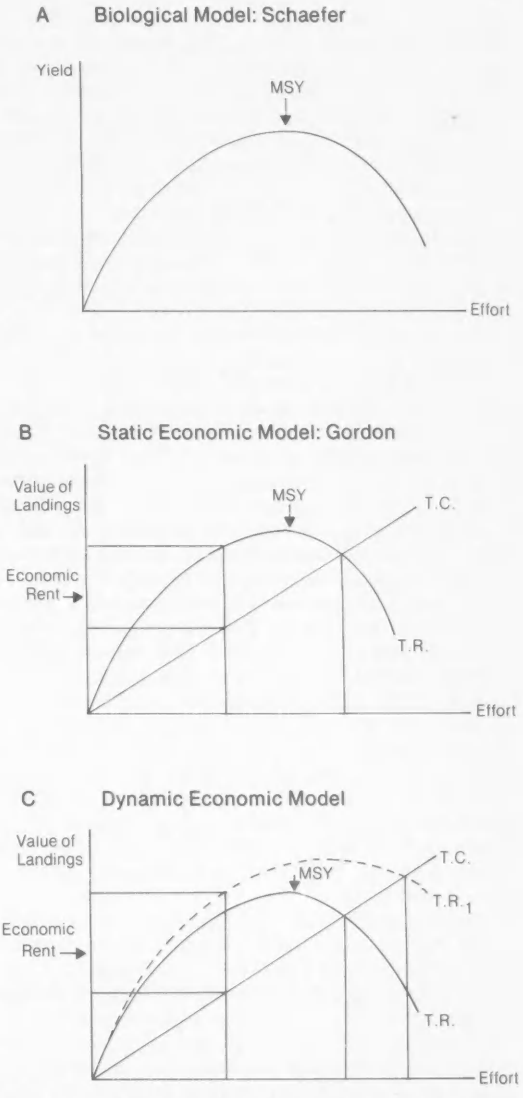

FIG. 2. A, Biological: Schaefer model — presents the production or yield function of an exploited fishery showing the MSY level. B, Static economic model: Gordon — the biological production function is converted to a total revenue function T.R. for fisheries and the total cost of fisheries exploitation depicted as a linear function of fishing effort, T.C. C, Dynamic economic model — dynamic changes due to increased prices are depicted resulting in changing the total revenue function from T.R. to $T.R_1$.

plied to fisheries by Schaefer, now popularly known as the Schaefer model (1953). This model for a single species shows a production or yield function to man's fishing effort as depicted in Fig. 2A. The underlying population responsible for this yield function grows when the natural rate of growth exceeds the reductions from the combined effects of natural mortality and man's fishing effort. It is the yield from fishing effort that the economist is interested in, not the size of the underlying population whose maximum size does not correspond with the highest yield (under Schaefer's logistic the yield is at its maximum when the population biomass is half its maximum size).

The Schaefer model shows that man's fishing effort, no matter how measured, for example the number of boats, fishermen, or days fished, can result in an increased yield with increased effort up to a maximum, the maximum sustainable yield (MSY), after which further increases in effort result in a lower yield brought about by the overexploitation of the resource. That is, in the exploitation of fisheries resources, there is an MSY beyond which increased effort imposes a strain on the reproductive capability of the population resulting in decreasing yields. This model and others like it influence biological resource management. Since this management's objective was conservation, the model indicated that fishing effort should not be allowed past the MSY level. This level was also erroneously considered the optimum economic level of exploitation by biologists. The rationale was that surely the level of effort that produced the highest catch and therefore the largest amount of food was certainly the best from society's standpoint. "Not necessarily so" said the economist, in fact it is hardly likely that this situation would be the best from society's standpoint. This can be demonstrated from the economic model which is really a bioeconomic one.

The economic model, first expounded by Gordon, was based on introducing economic variables to the biological model. This was done by turning the production yield function into a total revenue function and by introducing a total cost function, assumed to be increasing linearly with fishing effort, and which includes all costs such as alternative returns to the factors of production. To have a well-behaved total revenue function that corresponded to the physical production function, it was assumed that the cost per unit of fish landed would remain the same (an oversimplification in economics which will be taken care of later). In economics, the economic optimum occurs when net returns to the factors of production are greatest: when the marginal revenue equals marginal cost. Based on this, the economic optimum level of exploitation is shown in Fig. 2B.

The economic optimum in this model indicates that this is at a lower level of landings than the MSY. This is the level where the factors of production are most efficiently employed in that any incremental increase in since alternative returns are included in the costs, the factors of production are gaining returns commensurate with what could be obtained in other sectors of the economy. In other words, if there were no alternative sources of food in the economy but the fishery, and the demands for food were high, then it might make sense to exploit the resources up to their MSY levels. Otherwise, this is not the case because this constitutes a level where the returns to the factors of production are less than optimal, i.e. less than could be obtained in alternative opportunities.

Despite all the advantages of the economic optimum, fisheries exploitation generally does not take place at this level. Because fisheries resources are common property and subject to free entry, i.e. everyone who wishes to fish can fish, there is a tendency to dissipate the economic rent resulting in an equilibrium situation where total costs from fishing equal total revenues. This leads to both biological and economic disaster: the former exemplified by overfishing and lower sustainable yields, the latter by low returns and incomes and no rent from fishing.

Although the Gordon model outlined is static and simplistic and many refinements have been made in the economics literature since, its basic thesis that uncontrolled exploitation of a common property resource leads to overexploitation and poor economic returns has never been refuted. In fact, the recent history of commercial fisheries exploitation firmly attests to its relevance. However, the model does not take into account the dynamic nature of economic forces, for example, changes in prices due to increased demands for fish products, and fishing costs that do not necessarily occur at a constant rate. Thus, demand can increase prices and affect the total revenue curve in such a way that it does not resemble the original biological logistic curve. In fact, if a total revenue curve that takes into account increased prices is superimposed on the physical yield function of Fig. 2A, the model could look like the one depicted in Fig. 2C. This indicates that economic forces could lead to greater overexploitation of resources than the Gordon model shows. More sophisticated dynamic analyses by Scott (1955) and Clark (1971) have in fact indicated this to be the case.

It is not the purpose of this paper to pursue the dynamic economic analysis in detail. However, this analysis, which is concerned with "the rational allocation of resources over time" (Scott 1954), addresses itself to two key questions: (1) to what extent should there be investment (buildup) or disinvestment in the resource, and (2) the speed with which this investment or disinvestment should take place. This mainly re- revenues from their output does not result in a greater incremental increase in cost. At this level, the fishermen exploiting the resources are getting maximum returns (returns to labor and capital and rent from the resource). Society on the whole is better off because,

quires a capital theoretic approach involving the discounting of the future benefits and costs of fisheries exploitation for the purpose of maximizing the net present value of the resources. By using discount rates, which in effect measure how society regards or values present consumption in comparison with future consumption[2], the dynamic economic analysis indicates whether it makes sense to underexploit or overexploit the resources. Clark (1973) in fact showed that high discount rates could lead to the extinction of the resource. The dynamic economic analysis has not yet exerted any significant influence on fisheries management due to data constraints and limited mathematical computational methods. However, it really "fine tunes" and extends the operational principles of the static model. As a result, the analysis to follow will concentrate on the impact of the static economic model.

Impact of Economics on Fisheries Management

The static economic model exerted important influences on fisheries resource management in many parts of the world. The most recent and important in Canada has been the federal government exerting control on entry through license limitation programs in most fisheries, especially after obtaining management rights over the 200-mile Economic Zone. In just about every fishery where control on entry has been established, the economic benefits have followed fairly quickly. In the United States, jurisdictional problems between the federal and state governments have made the adoption of entry limitation difficult and there is still lively debate on the relative merits of free entry and license limitation.

The other important influence is that biologists, after at first having difficulty with the economic model, began to adopt it, but mainly for conservation reasons. Because in Fig. 2B the economic optimum was to the left of the MSY, it was thought that this level would ensure that overexploitation would not take place. Based on this model and the work of Gulland (Gulland and Robinson 1973) biologists came up with the $F_{0.1}$ rule defined as "the value of the fishing mortality at which the marginal yield per recruit (i.e. the addition to the total yield per recruit resulting from adding an additional unit of mortality) is 10% of the catch per recruit per unit mortality in a very lightly exploited stock. This has been referred to in some documents as "F_{opt}." (ICNAF Redbook, Part I, 1972). This rule has become the cornerstone of Canadian management of the 200-mile Economic Zone. As a result of its adoption, many biologists are convinced that they have come to terms with the economics since there is evidence in Canada's east coast fisheries that the $F_{0.1}$ level is reducing fishing costs and resulting in higher net revenues than would have occurred under MSY conditions (W. G. Doubleday, unpublished data).

The $F_{0.1}$ is, however, based on the static economic model, the Gordon model, from which economists have now gone beyond into the more dynamic models for reasons given earlier. For example, even if the $F_{0.1}$ rule results in improved economic returns these benefits would soon be dissipated in the absence of specific controls on effort.[3] This would occur either by more vessels entering the fishery or by the introduction of more costly technology to give vessels a competitive edge. For these reasons, economists have advocated effort control through a combination of licensing and economic measures such as license fees, access fees, royalties or taxes on landings. These are all designed to bring about a more rational use of fishing effort and to capture some of the economic returns for society which after all owns the resource.

There are therefore many differences in the approaches of biology and economics to commercial fisheries management. The acceptance of the management of commercial fisheries based on the "best use" principle entails that fisheries managers will be required to integrate biological and economic expertise to solve management problems. It would also necessitate a review of past resource management practices such as closed seasons, gear, and technology limitations, which have been imposed primarily for biological reasons, i.e. conservation purposes, and which prevented the economic exploitation of the resources. Although many of these measures might still be necessary for conservation purposes, it has been suggested (Sinclair 1978) they be kept to a minimum because of their adverse economic effects.[4] At the same time, economic measures might be difficult to implement because they might be disruptive to the industry and therefore unacceptable for political and social reasons.

Despite the factors mentioned above the main challenge for effective fisheries management is to choose the best tools or combination of tools to do the job. These should (1) encourage innovation and research into new methods, (2) be flexible to allow for proper

[2]A high discount rate indicates that society places a greater value on present consumption than on future consumption since as the discount rate is increased the present valuation of consumption in the future is decreased. The static Gordon model is based on a discount rate of zero which cannot be justified because it places no weights on present versus future consumption.

[3]It is thought that quotas based on the MSY or $F_{0.1}$ rule are effective effort control measures by themselves. This is not the case, even though quotas affect fishing effort in that they can reduce the number of days fished by the fleet; they do not, at least in the short run, result in a smaller fleet nor do they improve the economic performance on the fleet. On the contrary, quotas by curtailing fishing time lead to a longer tie up of the fleet and to lower economic returns.

[4]Sinclair's study is an excellent one for outlining some of the differences between the biological and economic approaches to fisheries management.

reaction to changes in economic and biological conditions, (3) have, ideally, the support of the majority of fishermen involved, and (4) be cognizant of the cost of management, i.e. administration, research, etc. (Anderson 1977, p. 151). Cost is important since this management should not only be effective in an operational sense but should be efficient in terms of attaining maximum benefits at lowest possible costs. This is the only way to ensure optimum benefits to society as a whole.

Discussion and Summary

This paper is a short analysis of the contributions of biology and economics to fisheries management. It points out that these two disciplines differ in their approaches to management with biology being concerned primarily with the conservation of resources and economics with the total economic benefits that can be generated from fisheries exploitation. However, fisheries management as stated by the "best use" principle involves a combination of both disciplines since the objective of this approach can be expressed as the maximization of socioeconomic returns subject to both biological and economic constraints. The main challenge to fisheries management is how to make the "best use" principle an operational concept. For this purpose, the paper identifies the areas of concentration for biological and economic expertise and the area where these two disciplines should interact in a management framework. This later area was identified as that of resource allocation because this allocation either for the commercial and sport fisheries or between different segments has to be based on socioeconomic considerations.

The paper demonstrates the great complementarity between biology and economics particularly in the development of the economic theory of fisheries, namely the static Gordon model which has its origins in the biological Schaefer-logistic model. The economic model indicated that because of free entry economic forces could lead to overexploitation in both a biological and economic sense, but the economic optimum, if effort could be controlled, would be at a level of exploitation less than the MSY. This led to biologists accepting this model and in an effort to make it operational devising the $F_{0.1}$ rule which has been applied to Canada's fisheries. This rule, however, based primarily on biological considerations cannot take into account dynamic economic forces and therefore does not control effort in a manner that would result in the "best use" of resources.

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ANDERSON, L. G. 1977. The economics of fisheries management. The John Hopkins University Press, Baltimore, Md. 214 p.

CLARK, C. W. 1971. Economically optimal policies for the utilization of biologically renewable resources. Math. Biosc. 12: 245–260.

1973. The economics of overexploitation. Science 181: 630–634.

ENVIRONMENT CANADA. 1976. Policy for Canada's commercial fisheries. Ottawa, Ont. 70 p. + appendices.

GORDON, H. S. 1953. An economic approach to the optimum utilization of fishery resources. J. Fish. Res. Board Can. 10: 442–447.

GULLAND, J. A., AND M. A. ROBINSON. 1973. Economics of fishery management. J. Fish. Res. Board Can. 30: 2040–2050.

ICNAF. 1972. International Commission for the Northwest Atlantic Fisheries, Standing Committee on Research and Statistics Proceedings. Redbook 1972, Part I: 87 p.

SANCHO, N. G. F., AND C. L. MITCHELL. 1975. Economic optimization in controlled fisheries. Math. Biosci. 27: 1–7.

SCHAEFER, M. B. 1953. Fisheries dynamics and the concept of maximum equilibrium catch. Proc. Gulf Caribbean Fish Inst., Sixth Annual Session, November, p. 53–64.

SCOTT, A. D. 1954. Conservation policy and capital theory. Can. J. Econ. Polit. Sci. 20: 503–513.

1955. The fishery, the objectives of sole ownership. J. Polit. Econ. 63: 116–124.

SINCLAIR, W. F. 1978. Management alternatives and strategic planning for Canada's fisheries. J. Fish. Res. Board Can. 35: 1017–1030.

WATT, K. E. F. 1968. Ecology and resource management. McGraw-Hill Book Company, Toronto, Ont. 450 p.

BOOK REVIEWS/COMPTES RENDUS

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Power Plant Entrainment. A Biological Assessment. *Edited by J. R. Schubel and Barton C. Marcy, Jr. Academic Press, Inc., New York, N.Y. 1978. x + 271 p. $16.00.*

The intended target for this book is the decision-makers who control or influence design of thermal generating stations. The book does not provide the panacea required for this method of electrical generation as it isolates entrainment (it was written by an ad hoc committee on entrainment) from the spectrum of environmental problems. Consequently, the book is effectively limited to decision-makers at the condenser cooling system level. Admittedly, creation of the *magnum opus* required to cure the ills caused by thermal electric generation is some time away but, as the authors point out, decision-makers *do* need the critical assessment of the effects of power plants, a part of which is adequately provided by this book.

Chapters with reviews of thermal effects of entrainment, effects of physical stress on entrained organisms, effects of biocides, and field studies are later combined in two chapters to yield recommendations and an evaluation of sets of conditions for the once-through cooling process. The committee do themselves an injustice by entitling the chapter "Selecting the Excess Temperature to Minimize the Entrainment Mortality Rate" as they rather effectively combine the interrelations among flow, temperature, and physical and chemical stress. Unfortunately, the formulae describing interplay of characteristics may cause the value of this particular chapter to be lost to those for whom it was intended. On the other hand, the chapter providing the conclusions and recommendations is clear and concise for those who only want the bottom line.

The other segments of the intended audience — scientists, funding agencies that support power plant research and environmentalists — will be rewarded, frustrated, informed, and at times confused. The book provides a good update and expansion of Coutant (1970), Saila (1975), and symposia proceedings even though segments are virtually identical to portions of earlier works. The redundancy, recognized in the preface, and repetitivity are frustrating, particularly when discrepancies over interpretation of references and presentation of facts (I noted four different ranges in total mortality for entrainable fish presented in the different chapters) are not resolved. Further, the difference in level of treatment impedes reading as the treatment appropriate to a review is interrupted by unnecessary detail of experimental history of test organisms and test procedures (frustration is at its peak in latter stages of the chapter "Thermal Effects of Entrainment"). Adequate editing would have greatly helped but the redundancy, repetitivity, and inconsistency will not deter the persistent reader and the new information, obscure references, and worthwhile interpretations are valuable to those interested in temperature, physical effects, biocides, and field assessments at power plants. The chapter "Cumulative Effects, a Field Assessment" (a total of 20 pages with 17 of these in table form) was a waste since virtually all the entries were dealt with elsewhere in terms of the factor causing mortality and the opportunity to analyze how mortalities at existing plants relate to mortality expected from the particular design and how the tendered recommendations could alter the situation was not taken.

The book is littered with recommendations. Many go beyond entrainment into behavioral response to effluents and bioconcentration of contaminants. The impact of the conclusions and advice is lessened since four chapters each provide their own isolated summary of research questions and recommendations, at times ignoring the interplay among factors controlling survival, and these are followed by a chapter of conclusions and recommendations. Confusion can be avoided for readers if they read only the last chapter and ignore recommendations provided with each chapter unless that subject is of particular interest.

The appendix, "Purgatorio," where the engineering description of the sequence of stresses during entrainment is paralleled with the story of an old fish that survived to an inexperienced larva is "cute" but unnecessary unless the audience is held in low esteem.

The book is well produced and the graphics are excellent. It provides a significant advancement beyond what is available to aid understanding the process of entrainment. Readers are enticed by the exhortation that once-through cooling is environmentally acceptable in some environments, particularly if operating conditions minimize entrainment mortality. These environments are never adequately described. Large lakes and rivers are recommended by the authors as bodies of water where once-through cooling is appropriate yet there is growing evidence that there, too, the cumulative effects of entrainment, impingement, and other environmental perturbations are severe. It is disappointing to see only one piece in the puzzle of where to place, how to design, and how to operate thermal generating stations and that only limited understanding of the ecosystem's response to the process of thermal electric generation is available.

COUTANT, C. C. 1970. Biological aspects of thermal pollution 1. Entrainment and discharge canal effects. C.R.C. Critical Review in Environmental Control 1: 341–381.

SAILA, S. B. ed. 1975. Fisheries and Energy Production. A Symposium. D. C. Heath and Co., Lexington, Mass. 300 p.

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Biology of benthic organisms. 11th European Symposium on Marine Biology, Galway, October, 1976. *Edited by B. F. Keegan, P. O. Ceidigh, and P. J. S. Boadan. Pergamon Press, Oxford, England. 1977. xxxiii + 630 p. $50.00.*

This volume presents a useful review of the current state of marine biology in Europe. Although there are a few articles by North Americans, the vast majority of the articles are by European scientists working in European habitats. The range of subject matter is broad and extends from abyssal depths to the upper littoral zone and from analysis of community structure to biochemical genetics to mechanics of filter, suspension, and deposit feeding. There are over 60 articles in the volume and what follows is a brief sampling of some of the articles that captured my interest.

Some articles hold interest for theoretical ecologists such as the demonstration by Faller-Fritsch that winkles may be either k- or r- strategists as circumstances require, the discussion by Moore of the factors contributing to the stability of communities, and the demonstration by Gosling and Wilkins that mussel genotype is correlated with microhabitat. There are also articles of a more practical, and perhaps cautionary, content such as the Andersin, Lassig, and Sandler article on the regulation of Baltic fauna by salinity and oxygen and the occasional exacerbation of these conditions by increased salinity and eutrophication. Of similar practical interest is Jephson and Gray's discussion of the invasion of Britain by *Sargassum muticum*. There are several articles (e.g. by Arntz and by Reise) on what can and cannot be expected from experiments with cages in the field. Several of the papers on community structure analysis employed multivariate techniques effectively (e.g. Chardy and Glémarc; Desbruyères and Guille; Eagle and Hardiman; Knight-Jones and Nelson-Smith; Rex). Two articles showed by quite different means that sediment type may be less important than previously expected in controlling community structure (Richter and Sarnthein; Tyler). For those interested in exotica, there is a fascinating article by George on the ecology of pogonophores which includes photographs and descriptions of the behavior and biology of living specimens. Finally, there is the exhortation by H. Barnes and M. Barnes to consider the roles of meroplankton as both consumers and prey in studies of the trophodynamics of neritic ecosystems.

This summary of the contents should give the reader an indication of the variety of material available. At that price, most readers may not find it necessary to own a personal copy, but certainly many libraries will require one for reference.

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(R170)

Development of Fishes of the Mid-Atlantic Bight. An Atlas of egg, larval and juvenile stages. Vol. I — Acipenseridae through Ictaluridae. 366 p., 199 fig. compiled by Philip W. Jones, F. Douglas Martin, and Jerry D. Hardy Jr. Vol. II — Anguillidae through Syngnathidae. 458 p., 245 fig. compiled by Jerry D. Hardy Jr. Vol. III — Aphredoderidae through Rachycentridae. 394 p., 230 fig. compiled by Jerry D. Hardy Jr. Vol. IV — Carangidae through Ephippidae. 314 p., 175 fig. compiled by G. David Johnson. Vol. V — Chaetodontidae through Ophidiidae. 340 p., 178 fig. compiled by Ronald A. Fritzsche. Vol. VI — Stromateidae through Ogcocephalidae. 416 p., 205 fig. compiled by F. Douglas Martin and George E. Drewry. *Fish and Wildlife Service, U.S. Department of the Interior, U.S. Government Printing Office. 1978. $62.50 U.S., hardbound.*

The goal of this six volume monograph was to synthesize the world's literature on fishes occurring in the Mid-Atlantic Bight which includes the fresh and coastal waters of New Jersey, Virginia, Delaware, and Maryland out to the 100-fathom line, to help in identifying young fish eggs and larvae, and to indicate gaps in knowledge. The accurate identification of larval fish as part of our natural resources becomes more important as man constructs various kinds of power plants and continues to further modify the aquatic environment.

As anyone knows who has tried to identify fish larvae, the relevant literature is widely scattered in numerous journals. Bringing this information together will greatly ease the problem for workers in the Atlantic Bight and will be useful for others working in adjoining regions. For example, of the 341 species treated at least 42% occur in fresh waters of Canada. This compilation will be welcomed by many students and identifiers who will develop more confidence than before these books were available. However, our past experience has shown that many misidentifications will continue to be made and it will still be necessary for persons using these books to obtain aid from persons familiar with hard-to-identify species.

Workers along the Atlantic coast have had a variety of indigenous works of variable quality to aid them in their identifications but none are as complete or accurate as the present compilation. Among the most notable are Alexander Agassiz's and John A. Ryder's early works in the latter part of the 19th century, Kuntz and Radcliffe (1917), Dannevig (1918), Hildebrand and Schroeder (1928), Hildebrand and Cable (1938), Fish (1932), Merriman and Sclar (1952), and Bigelow and Schroeder (1953). A number of report-type studies concerning the identification of larval fishes have been produced recently but most are no better nor no worse than the publications from which they were derived.

A glance through these six volumes reveals a fascinating diversity of structures such as the flap-like barbels of flying fishes, the filamentous eggs of needle fishes, the transparent leaflike eel larvae, the rostral cement glands of the longnose gar, etc. A great deal of information about the early life of fishes can be gleaned from these volumes, and it is to be hoped that this information will stimulate further research.

Every volume starts with the same glossary of terms and diagrammatic illustrations that define the words used and each has its own table of contents, bibliography, and, hidden behind the bibliography, an index to common and scientific names; thus each volume is a self-contained unit. Literature up to and including 1977 is cited but there is no overall index. Species are arranged alphabetically within families which are themselves arranged, unfortunately, by the out-of-date American Fisheries Society (1960) list; it would have been preferable to follow a more recent classification, preferably Nelson's (1976) *Fishes of the World*.

Most of the material, but not all, is drawn from the

literature; therefore, species accounts vary markedly according to how much study has been devoted to the species. The text for each species is divided into adult description, distribution and ecology, knowledge of spawning, descriptions of egg stages, details of egg development, descriptions of yolk-sac larvae stages, descriptions of larval stages, descriptions of juveniles, knowledge of age and growth, and age and size at maturity. Extensive superscripts in the text lead to an abbreviated citation at the end of the species account with a full citation at the end of the volume. An added section concerning taxonomic problems with species or subspecies would have been useful. With such a large undertaking to compile so much confusing data, there are bound to be some errors; for example, in Vol. I, p. 246, predorsal myomeres are used in place of preanal myomeres, but we found surprisingly few such errors in reading and comparing the descriptions of several species.

Each species is accompanied by at least a drawing of the adult as well as numerous illustrations of egg stages, larval stages, and juveniles when available. The carp, for example, has 105 illustrations taken from numerous sources. Most drawings are reproduced from original sources which avoids error in reproduction; a few have been redrawn to correct known errors in originals and even a few new drawings are included. The illustrations, however, are still a compilation of numerous artistic endeavours. Such a compilation makes it difficult, if not impossible in some cases, to separate accurately one described egg or larva from another. Leafing quickly through any of the six volumes one can see good and bad illustrations. It is recommended that illustrations of larval stages to be published in the future should avoid artistic shading and the inclusion of myomeres, except for exceptional circumstances, because the added lines, dots, and shadings are confused with the more important pigment details. Most larvae are transparent and the pigmentation on an illustration shows as either a dark dot or line. Thus any artistic endeavour by an over-enthusiastic artist will be confusing to a person trying to compare a preserved larvae with an illustration. All such lines and artistic shadings should be made useful for the purposes of accurate representation.

It is encouraging to see the compilers admit the possibility of error in some species descriptions, i.e. *Seriola dumerili*, Vol. IV, p. 79, and it is hoped that users of this publication will take notice of those when assigning names. Workers should be encouraged to publish corrections in the future. We sincerely hope that the appearance of these volumes will not discourage researchers from publishing thorough descriptions of these and other larvae from waters along the Atlantic coast of North America when further information becomes available. It is still extremely difficult to separate closely related species with these volumes although in some instances a preliminary attempt was made, i.e. Gadidae, Clupeidae, and others. There is a need for a synthesis of certain closely related forms, taking into account pattern of pigmentation, meristics, osteology, stage of development, and possibly details of pigmentation and/or morphology that have not been described yet. As an example, Hardy (Vol. III, p. 69) stated that Fritzsche and Johnson have successfully separated white perch, *Morone americana*, and striped bass, *Morone saxatilis*, by internal pigmentation and bone and cartilage development. These ideas along with the detailed morphological work of Moser and Ahlstrom (1970) could be incorporated into the work of east coast researchers. We also suggest that the appearance of this work makes it possible for writers to produce popular articles on the early life of some of these interesting fishes.

It is nice to see that the U.S. Government was willing to spend a great deal of money to have these six volumes written and published. At $10.00 per volume these grey hardbound books are indeed a bargain compared to the normal price of such books. We sincerely hope that the funds spent will aid the North American consulting firms to increase their quality of larval fish identifications. A hearty thanks to the Power Plant Project, Office of Biological Services, Fish and Wildlife Service, U.S. Department of the Interior, for their encouragement and financial support. These volumes serve as a notable memorial to Dr Romeo Mansueti who envisioned the project in the early 1960s but who passed away before seeing its completion. They will stand as an invaluable contribution to the biology of North American fishes and hopefully will stimulate further interest in the study of early life history.

AMERICAN FISHERIES SOCIETY. 1960. A list of common and scientific names of fishes from the United States and Canada, Am. Fish. Soc. Spec. Publ. No. 2, 2nd ed. 102 p.

BIGELOW, H. B., AND W. C. SCHROEDER. 1953. Fishes of the Gulf of Maine. U.S. Fish and Wildlife Ser. Bull. 53(74): 1–577.

DANNEVIG, A. 1918. Fish-eggs and larvae, p. 1–80. *In* J. Hjort [ed.] Canadian Fisheries Expedition 1914–15, in the Gulf of St. Lawrence and Atlantic Waters of Canada. Dep. Naval Serv. Can., King's Printer.

FISH, M. P. 1932. Contributions to the early life histories of sixty-two species of fishes from Lake Erie and its tributary waters. U.S. Bur. Fish. Bull. 47(10): 293–398.

HILDEBRAND, S. F., AND W. C. SCHROEDER. 1928. Fishes of Chesapeake Bay. U.S. Bur. Fish. Bull. 53(Pt. I): 1–388.

HILDEBRAND, S. F., AND L. E. CABLE. 1938. Further notes on the development and life history of some teleosts at Beaufort, N.C. U.S. Bur. Fish. Bull. 48(24): 505–642.

KUNTZ, A., AND L. RADCLIFFE. 1917. Notes on the embryology and larval development of twelve teleostean fishes. U.S. Fish. Doc. 849: 44 p.

MERRIMAN, D., AND R. C. SCLAR. 1952. The pelagic fish eggs and larvae of Black Island Sound. Bull. Bingham Ocean. Coll., Yale Univ. 13(3): 165–219.

MOSER, H. G., AND E. H. AHLSTROM. 1970. Development of lanternfishes (Family Myctophidae) in the California current. Part I. Narrow-eyed Species. Bull. Los Angeles City Museum Nat. Hist. Soc. 7: 1–145.

NELSON, J. S. 1976. Fishes of the world. John Wiley & Sons, Inc., New York, N.Y. 416 p.

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(R235)

Anatomie de *Latimeria chalumnae*. Tome III. Appareil digestif — appareil respiratoire — appareil urogénital — glandes endocrines — appareil circulatoire — teguments — écailles — conclusions générales. *J. Millot, J. Anthony and D. Robineau. Editions du Centre national de la Recherche scientifique, 15, quai Anatole — France, 75700 Paris. 1978. 198 pp., 86 pl. incl. 1 col. pl., 44 text-fig incl. 2 folding, 34.5 x 27.5 cm. 450 francs (about $127 Canadian).*

This magnificent volume completes publication of years of study on the living fossil *Latimeria chalumnae*, which began with volume 1, in 1958. The green leather binding with gold lettering, quality matte paper and splendid photographic plates combine with an authoritative text to make a scholarly monograph that will be a landmark in the history of anatomy and continues the ichthyological tradition of Cuvier and Valenciennes. Classical anatomical techniques of dissection are employed with scanning electron microscopy, microradiography, injection of radio-opaque fluids with macroradiography, and cinematography to produce an in-depth view of anatomy.

This volume describes the respiratory, urogenital and circulatory systems, endocrine glands, skin, and scales. About one third is devoted to the circulatory system. A résumé with general conclusions summarizes and updates finding in previous volumes as well as the present one. A 24-page bibliography organized by subject, list of abbreviations, and 86 plates complete this volume.

Some of the anatomical discoveries may be mentioned. The spiracular canal, open in rhipidistians, is closed in *Latimeria*. A vestigeal ductus pneumaticus connects with the floor of the pharynx as in lungfishes but is not open, the lung itself being charged with fat instead of gas. The spiral valve in the intestines is of the cylindrical type found in carcharid sharks, describing 17 clockwise turns. Mauthner's axons, the giant neurons linking sensory, cerebellar, and motor escape systems in many fishes, are missing. The brain, which fills the cranial cavity in young specimens, occupies only 1% of it in adults. Unlike most fishes pleural ribs are lacking. The spermatozoa are uniflagellate unlike those of the dipnoans and are reported to be most similar to those of selachians. Up to 19 orange-sized eggs are reported free in the body cavity of females. These are the biggest eggs without shells in fishes. The coelacanth is now known to give birth to live young. The kidney is unique in that it is ventral instead of dorsal, displaced there by the dorsal fat-filled lung, and in being single instead of paired. On the other hand, there are two, instead of the usual one, urinary bladders.

The monograph does not restrict itself to simple anatomical description but makes functional interpretations and comparative comments. The functional biomechanical relationships of the feeding and respiratory apparatus are discussed in relationship to measurements and movements observable in freshly thawed specimens. The fore and hind parts of the cranium are separate, linked by the notochord and subcranial muscles (which means that its brain is flexible anatomically, if not conceptually). The forecranium can be flexed downwards 15–20° during respiration or preliminary to feeding using the subcranial muscle, returning to its previous position through the elasticity of the notocord. One would have liked to have seen comments on the functioning of the paired gular plates since there is only one other group of living fishes that has a pair.

Some observations illuminate the biology of the coelacanth. The very moderate cardiac capacity and the very low relative area of the 4½ gills (lower than most fish) indicate its sedentary life-style. The coelacanth preys mostly on fishes up to 10–20% of its own length, but also on crustaceans. Its cardiovascular capacity suggests that it lies in wait for these prey. The sensitivity of the eyes is high but the rarity of cones bespeaks of poor color sensitivity. The light is feeble at this depth and as captures are usually made at night it would seem likely that other senses help locate the prey. The reviewer would again like to suggest the hypothesis (McAllister 1971. Odyssey Collection (1): 10) that the mysterious rostral organ in the snout of the coelacanth is an electroreceptor. Recent work by K. J. Rose found that sharks bit live electrodes in preference to tubes dispensing fish blood, strongly suggesting electroreceptivity for the ampullae of Lorenzini located in their rostra, the latter resembling the coelacanth's rostral organ. This and a new hypothesis, proposed by Thompson (1977, p. 247–270 in "Problems in vertebrate evolution," Academic Press, Inc., New York, N. Y.), that the pore–canal system in the dermal skeleton functions as an electroreceptor becomes more attractive in view of Millot, Anthony, and Robineau's observation that individual taste organs are absent in the coelacanth.

To the authors we are grateful for the feast of mind and eye they have provided for anatomists, systematists, students of evolution, and biology. They deserve congratulations for bringing to completion their long, devoted task. It is hoped that the Centre national de la Recherche scientifique and the Musée national d'Histoire naturelle will receive the support so well deserved for continuing studies in domaines of natural history. The English-speaking reader may find J. Vaillancourt's *Lexique anglais–français termes technique à l'usage des biologistes* published by the University of Ottawa useful in reading the *Anatomie de Latimeria chalumnae.*

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(R245)

Books Received/Livres reçus[1]

January 1979

*__Die Binnengewässer Band XXVII. Das Zooplankton der Binnengewässer. Teil 2.__ *Friedrich Kiefer and Geoffrey Fryer. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart. 1978. vii + 380 p. DM108.*

*__Les substances organique naturelles dissoutes dans l'eau de mer.__ *(Colloque du groupement pour l'avancement de la biochimie marine.) Réalisation du volume coordinée par Hubert J. Ceccaldi. Centre National de la Recherche Scientifique, Paris. 1978. iii + 270 p. 85.00 F.*

[1]Entries in this list *not* marked by an asterisk are available for review by interested readers for a 3-month period from the time of publication of the list. For information, please write to

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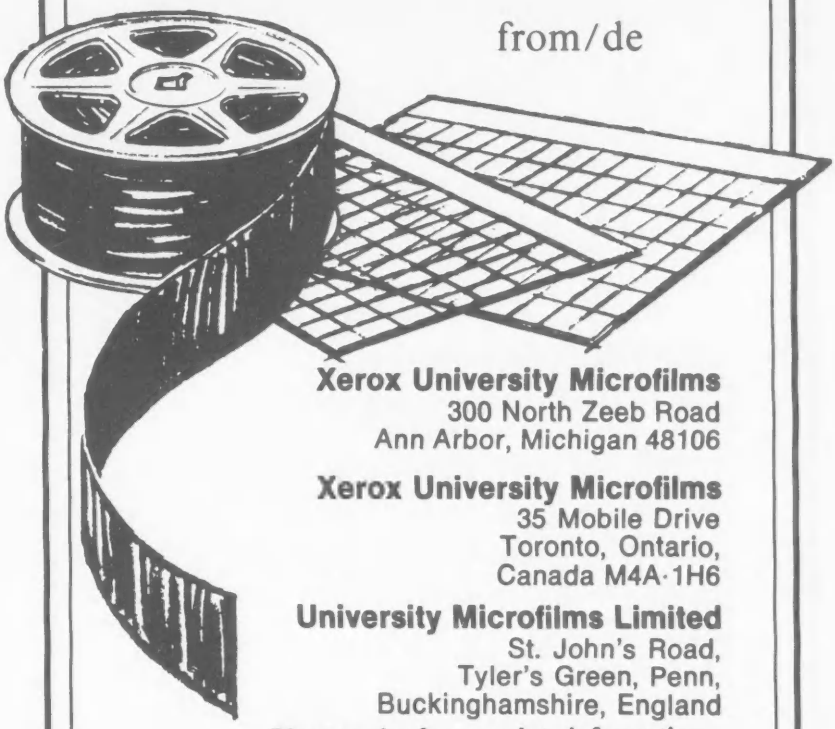

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Volume 36, No. 6, June 1979

Volume 36, nº 6, juin 1979

Physiological and biochemical aspects of ozone toxicity to rainbow trout (*Salmo gairdneri*) **G. A. Wedemeyer, N. C. Nelson, and W. T. Yasutake** 605–614

Physiology of salinity-dependent naphthalene toxicity in *Fundulus heteroclitus* **W. M. Levitan and M. H. Taylor** 615–620

Acute ammonia toxicity and ammonia excretion in rainbow trout (*Salmo gairdneri*) **B. A. Hillaby and D. J. Randall** 621–629

Karyotypic and evolutionary relationships of the Yellowstone (*Salmo clarki bouvieri*) and west-slope (*S. c. lewisi*) cutthroat trout **E. J. Loudenslager and G. H. Thorgaard** 630–635

Plaque-forming cells and humoral antibody in rainbow trout (*Salmo gairdneri*) induced by immersion in a *Yersinia ruckeri* O-antigen preparation **D. P. Anderson, B. S. Roberson, and O. W. Dixon** 636–639

Depression of pH in lakes and streams in central Ontario during snowmelt **D. S. Jeffries, C. M. Cox, and P. J. Dillon** 640–646

Population dynamics of spiny dogfish (*Squalus acanthias*) in British Columbia waters **C. C. Wood, K. S. Ketchen, and R. J. Beamish** 647–656

Phytoplankton ecology of the Strait of Georgia, British Columbia **J. G. Stockner, D. D. Cliff, and K. R. S. Shortreed** 657–666

Notes

Intertidal catastrophes: effect of storms and hurricanes on intertidal benthos of the Minas Basin, Bay of Fundy **R. K. Yeo and M. J. Risk** 667–669

Decrease in mercury content of fishes in Ball Lake, Ontario, since imposition of controls on mercury discharges **F. A. J. Armstrong and D. P. Scott** 670–672

Role of tubificid worms on nitrogen transformations in stream sediment **L. Chatarpaul, J. B. Robinson, and N. K. Kaushik** 673–678

Carbon isotope ratios of particulate organic matter in the Gulf of St. Lawrence **F. C. Tan and P. M. Strain** 678–682

Composition of granules from kidneys of rainbow trout (*Salmo gairdneri*) with nephrocalcinosis **D. C. Gillespie and R. E. Evans** 683–685

Rate of loss of ammonia from water to the atmosphere **R. R. Weiler** 685–689

Chronic exposure of trout to waters with naturally high selenium levels: effects on transfer RNA methylation **I. I. Kaiser, P. A. Young, and J. D. Johnson** 689–694

Density estimates of *Mysis relicta* in Lake Michigan **N. E. Grossnickle and M. D. Morgan** 694–698

Perspectives

Bioeconomics of commercial fisheries management **C. L. Mitchell** 699–704

Book Reviews/Comptes rendus: Power plant entrainment. A biological assessment (J. R. Schubel and B. C. Marcy [ed.]) **J. R. M. Kelso**; Biology of benthic organisms. 11th European Symposium on Marine Biology, Galway, October 1976 (B. F. Keegan, P. O. Ceidigh, and P. J. S. Boadan [ed.]) **J. R. Sibert**; Development of fishes of the Mid-Atlantic Bight. An atlas of egg, larval and juvenile stages **D. J. Faber and D. E. McAllister**; Anatomie de *Latimeria chalumnae*. Tome III. Appareil digestif — appareil respiratoire — appareil urogénital — glandes endocrines — appareil circulatoire — téguments — écailles — conclusions générales (J. Millot, J. Anthony, and D. Robineau) **D. E. McAllister** 705–708

Books received/Livres reçus 708

Coming next month/À paraître le mois prochain 709

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